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THE COGNITIVE EFFECTS OF DOPAMINERGIC LESIONS TO THE ANTERIOR CINGULATE CORTEX

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ABSTRACT

THE COGNITIVE EFFECTS OF DOPAMINERGIC LESIONS TO THE ANTERIOR CINGULATE CORTEX

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The anterior cingulate cortex (ACC) has been shown to activate when situations are in conflict, when determining the relevance of stimuli, and error processing. Dopaminergic projections to the ACC are hypothesized to facilitate the integration of incoming stimuli with error signals to select and maintain the optimal task set, reducing the liability to distraction. In previous work, rodents with excitotoxic lesions to the ACC showed increased susceptibility to distraction when a complex stimulus contained a stimulus dimension with a prior reward history. In an expansion of this work, following dopaminergic lesions to the ACC rodents were tested in an attentional set shifting task and a sustained attention task. In experiment 1, all subjects showed increased susceptibility to distraction, and either an inability to update reinforcement contingencies or an inability to overcome the increased susceptibility to distraction. Experiment 2 explicitly tested the ability of subjects to filter novel distractors with no prior reinforcement history, as well as the ability to update reinforcement contingencies without distractors present. Dopamine lesions did not increase susceptibility to distraction from novel distractors and did not globally impair the ability to update reinforcement contingencies. All males showed an ability to adjust to changes in reinforcement regardless of the delay, females, however, showed a specific inability to adjust only when reinforcement was delayed 2s. The difference in males and female's ability to adjust to changes in reinforcement in the sustained attention task requires further research. Together the experiments suggest that dopamine dysfunction in the ACC is sufficient to increase susceptibility to distraction when complex stimuli dimensions have a prior reinforcement history.

INTRODUCTION

Converging evidence suggests that dopamine in the anterior cingulate cortex (ACC) plays a crucial role in maintaining or adjusting strategies to achieve overarching goals. The ACC in humans has been linked to conflict monitoring (Bush et al., 1999; Erickson et al., 2004), error processing (Amiez, Joseph, & Procyk, 2005; Gehring, Goss, Coles, Meyer, & Donchin, 1993; Laurens et al., 2003; Scheffers, Coles, Bernstein, Gehring, & Donchin, 1996), determining the relevance of complex stimuli and filtering salient distractors (Jazbec et al., 2007). ACC dysfunction in humans has been linked to several neuropsychiatric disorders such as Attention Deficit Hyper Activity Disorder (ADHD) (Amico, Stauber, Koutsouleris, & Frodl, 2011; Bledsoe, Semrud-Clikeman, & Pliszka, 2013; Kollins, Lane, & Shapiro, 1997; Seidman et al., 2006), Obsessive-compulsive disorder (OCD) (Olver et al., 2010; Perani et al., 1995), and Schizophrenia (Dolan et al., 1995; Pantelis et al., 1997). While these disorders have been hypothesized to occur in part due to a dysfunction of the dopaminergic system in the ACC (Abi-Dargham & Moore, 2003; Dolan et al., 1995), this is not explicitly examinable in humans.

Rodent models allow for the assessment of cognitive failings associated with specific attributes of human neuropsychiatric disorders. The increased susceptibility to distraction seen in patients with schizophrenia (Demeter, Guthrie, Taylor, Sarter, & Lustig, 2013; Jazbec et al., 2007; Pantelis et al., 1999), has been seen in rodents following non-specific lesions to the ACC (Newman & McGaughy, 2011). The error processing signals which are depressed in ADHD (Tripp & Brent, 1999; Umemoto, Lukie, Kerns, Müller, & Holroyd, 2014), and exacerbated in OCD (Santesso, Segalowitz, & Schmidt, 2006), are elicited from the ACC in rodents (Hyman,

Holroyd, & Seamans, 2017; Hyman, Whitman, Emberly, Woodward, & Seamans, 2012; Warren, Hyman, Seamans, & Holroyd, 2015) and humans (Amiez et al., 2005; Crottaz-Herbette & Menon, 2006; Scheffers et al., 1996). The cognitive symptomology of schizophrenia and ADHD is thought to be due to a hypofunction of the dopamine system in the ACC (Abi-Dargham & Moore, 2003; Chen et al., 2016; MacKenzie et al., 2018), where the inverse has been seen in OCD (Olver et al., 2010). Dopamine in the ACC may be a crucial factor in integrating complex stimuli and error signals to determine which dimensions of a stimulus or task should be attended, and which should be filtered when a change in global strategy is needed.

Dopamine projections to the ACC have been postulated to modulate the ability to recognize and integrate unexpected changes in reinforcement only when a change in global strategy, also known as a task set (Sakai, 2008), is needed (Hollerman & Schultz, 1998; Holroyd & Coles, 2002; Schultz, 1997). Dopamine neurons in non-human primates have been shown to increase firing rates in the ventral tegmental area (the origin of the mesocortical and mesolimbic dopamine pathways) when reward arrives but is not expected and to decrease when expected reward is omitted (Schultz, 1997). These neurons are also responsive to changes in reinforcement delivery timing if a reward is early, late, or omitted (Hollerman & Schultz, 1998). Previous work has shown that non-specific lesions to the ACC in rodents produced an increased susceptibility to distraction only when irrelevant attributes of a complex stimulus have a prior reinforcement history (Newman & McGaughy, 2011). This suggests that the ACC may be important for stabilizing the current task set, where the integration of error feedback is utilized to stabilize (Robbins, 2005) and implement overarching strategies for optimal reward (Holroyd & Umemoto, 2016). The ability to produce or integrate error feedback may rely on the dopamine projections to the ACC, which the excitotoxic lesions could not determine.

In the present study, we expand on previous work in three crucial ways. First, this study aims to identify the underlying neurochemical basis for the results seen in the Newman and McGaughy 2011 study. Here, we depleted dopamine in the ACC, which allowed for the assessment of the role of dopamine in the ACC in filtering previously rewarded attributes of a complex stimulus using an attentional set shifting task (Birrell & Brown, 2000). Second, the current study incorporated both novel distractors with no previous reinforcement history as well as changes to reinforcement contingencies in a well-validated task of sustained attention (Demeter et al., 2013; McGaughy, Kaiser, & Martin, 1996; McGaughy & Sarter, 1995; Newman & McGaughy, 2008b, 2011). Novel distractors were used to determine if DA lesions to the ACC increase distractibility to all stimuli or produces a more specific distractibility from the elements of a complex stimulus that have a reinforcement history. The changes in reinforcement timing allow for the assessment of the ability to adjust to changes in reinforcement, without the presence of distracting stimuli. And third, as the previous study only included male subjects, a cohort of female subjects was included in the present study. As no sex differences have been seen in adolescent (McGaughy data, unpublished) and adult rodents (McGaughy & Sarter, 1999; Murphy, McGaughy, Croxson, & Baxter, 2016) in either the attentional set shifting task or the sustained attention task, none were expected in the present study. However, the inclusion of females in our study ensures the greater generalizability of our results.

CHAPTER I

BACKGROUND AND RATIONALE

Functionally Distinct Regions of the Rodent Medial Prefrontal Cortex

The ACC shows structural and functional differences in humans with neuropsychiatric disorders, such as ADHD (Amico et al., 2011; Bledsoe et al., 2013; Seidman et al., 2006; Umemoto et al., 2014), and Schizophrenia (Chen et al., 2016; Goldberg, Berman, Mohr, & Weinberger, 1990; Laurens, Kiehl, Ngan, & Liddle, 2005; Pedersen et al., 2012).While human models have shown the ability to assess changes in neural activity specific to the ACC (Bush, Luu, & Posner, 2000; Mohanty et al., 2007; Peterson & Posner, 2012; Stevens, Hurley, & Taber, 2011), they are unable to examine the neurochemistry underlying these functional increases or decreases in activity. To assess this, animal models must be used. In the five-choice serial reaction time tests (5-CSRTT), pregenual ACC lesions produced a decrease in accuracy, but not a decrease in inhibitory control, showing differentiation between infralimbic (IL)/prelimbic (PL), function, and ACC function (Chudasama et al., 2003). In the 5-CSRTT animals are presented a target stimulus in one of five spatial locations in an operant box fitted with five chambers (Robbins, 2002; Turner, Peak, & Burne, 2015). After the presentation, a 5s fixed inter-trial interval occurred, after which the back-panel light turns on for 0.5 seconds, indicating the ability to make a response. Within a 5s limited hold, nose pokes to the previously illuminated hole result in food reward (hit), while a non-previously illuminated hole results in no food (miss), and no

response is reported as an error of omission. Any nose pokes made during the ITI count as impulsive (premature) responses. Any responses made to the previously illuminated hole are reported as compulsive, perseverative errors (Chudasama et al., 2003; Chudasama & Robbins, 2004). Lesions to the ACC did not increase premature or perseverative responses but increased misses compared to controls. Lesions to the IL/PL produced increases in premature responses, and orbitofrontal lesions produced increased perseverative errors (Chudasama et al., 2003).

In the 5CSRTT accuracy is used as a measure of sustained and spatially divided attentional capacity (Bari, Dalley, & Robbins, 2008; Robbins, 2002). The task requires subjects to detect brief visual stimuli presented in a spatially unpredictable manner, requiring subjects to maintain attention to all five possible stimulus locations (Chudasama et al., 2003; Robbins, 2002). Subjects with lesions to the ACC show a lower accuracy compared to sham-lesioned subjects in the 5CSRTT (Chudasama et al., 2003). However, when an elongated and variable ITI (9±3s) was introduced, ACC lesioned subjects' accuracy did not change where sham lesioned subjects' performance declined, showing that the ACC lesioned subjects were not utilizing the 5s ITI to determine when to attend to the five light ports. These results suggest a decrease in attentional capacity following the ACC lesions, as well as an inability to exploit the temporal predictability when the ITI was consistent (Chudasama et al., 2003; Passetti, Chudasama, & Robbins, 2002; Robbins, 2002). The lack of perseverative errors indicates that ACC lesioned subjects did not continue to select ports which had been rewarded in the previous trial, unlike the orbitofrontal cortex (OFC) lesioned subjects (Chudasama et al., 2003), indicating that the rodent ACC plays a functionally distinct role from neighboring brain regions.

Rats with lesions to the ACC have increases in distractibility to previously reinforced attributes of a complex stimulus in an Intradimensional/Extradimensional set shifting (ID/ED)

task (Newman & McGaughy, 2011). In the ID/ED, task-specific cognitive deficits can be examined separately, based on the stage of the task in which subjects show deficit (Birrell & Brown, 2000; Downes et al., 1989). The task also utilizes complex stimuli that require the ability to determine the relevant dimension of a stimulus and ignore the other dimensions (Birrell & Brown, 2000). The task also employs a total changeover design in the stimuli, such that subjects need to apply the knowledge of the relevant dimensions and irrelevant dimensions (an attentional set) to the novel stimuli (Birrell & Brown, 2000). In the ID/ED task animals were able to form and use an attentional set, however, they were particularly susceptible to distraction when attributes of a complex stimulus had previously generated a reward response (Newman & McGaughy, 2011). In the 5CSRTT, the measure of accuracy is based on an ability to sustain attention across spatially divided locations over long periods of time (Chudasama et al., 2003; Passetti et al., 2002; Robbins, 2002). In the ID/ED task, the accuracy of selection is based on an ability to determine and implement the choice of a relevant stimulus dimension (Birrell & Brown, 2000). Lesions to the ACC produced an uncoordinated selection of ports in the 5CSRTT (Chudasama et al., 2003; Passetti et al., 2002), and increased susceptibility to distraction, but preserved ability to form, use, and shift an attentional set in an ID/ED task (Newman & McGaughy, 2011). Together, these results suggest that dysfunction of the ACC may lead to an increased susceptibility to distraction.

The ACC, IL/PL, and OFC in rodents have been shown to be functionally distinct. Non-specific lesions to IL/PL increased perseverative errors in the 5CSRTT (Chudasama et al., 2003). Non-specific lesions of rodent OFC have an increased inability to update reinforcement contingencies, showing perseverative errors in the 5CSRTT (Chudasama et al., 2003) and in reinforcement reversals in the ASST (Alexander, Tait, & Brown, 2012; Tait & Brown, 2007).

Non-specific lesions to the ACC have shown a decrease in attention, but not an increase in perseverative errors in the 5CSRTT (Chudasama et al., 2003), as well as increased susceptibility to distraction from previously reinforced elements of a complex stimulus in an ID/ED task (Newman & McGaughy, 2011). These functions have also been shown to be neurochemically distinct. Noradrenergic, but not cholinergic lesions of the IL/PL resulted in perseverative responses to the same dimension of a complex stimulus when it was no longer predictive of reinforcement (McGaugh y, Ross, & Eichenbaum, 2008). This cognitive rigidity seen from the noradrenergic lesions was reversed by the administration of the norepinephrine reuptake inhibitor atomoxetine (Newman & McGaughy, 2008a). However, cholinergic lesions to IL/PL produced an increased susceptibility to distraction from cross-modal stimuli (Newman & McGaughy, 2008b). The administration of dopamine agonists in low performing rodents produced an increase in accuracy in the 5CSRTT (Chudasama & Robbins, 2004), possibly indicating a role for dopamine in the ACC in modulating the deficits seen in the non-specific ACC lesion (Chudasama et al., 2003). However, the cognitive effects of dopaminergic lesions to the ACC remains a gap in the literature.

Error Processing

The ability to detect an error is important to be able to make changes in behavior to achieve the best possible outcome. The ACC is critical for integrating error, conflict, and reward information into working memory (Rushworth & Behrens, 2008). Studies in non-human primates have shown that lesions to ACC sulcus (ACCs) produce difficulty in utilizing past reward history to make beneficial choices (Kennerley, Walton, Behrens, Buckley, & Rushworth,

2006). In this study, monkeys had to choose between turning or lifting a joystick for a reward. Responses needed to be sustained for 25 rewarded movements; then, the contingency for reward switched, and the other movement elicited reward. Monkeys with ACCs-Lx were less likely to make a correct movement following a rewarded movement (Kennerley et al., 2006). In the second task, the win-stay, lose-shift strategy for success was eliminated with a ratio of reward probability design. Here, monkeys needed to try both movements to determine which movement would most often elicit reward. ACCs-Lx animals took longer than sham-Lx animals to reach the optimal movement strategy for all of the ratios tested (Kennerley et al., 2006). The authors posit that ACCs-Lx monkeys are unable to utilize previous reinforcement history to make ideal decisions.

Action-guided decision making in non-human primates has been shown to be dependent on ACC activation. Luk and Wallis (2013) aimed to differentiate the regions involved in action-outcomes and stimulus-outcomes. They developed an analogous, two-step decision making task that could be used to separate action-outcomes from stimulus-outcomes. In the first phase "sampling," monkeys are either shown a picture or need to move a lever to the right. After this, the juice was given. Following a 1s delay, a second image, or movement to the left occurred, followed by a different juice. The second phase "choice" consisted of a side-by-side presentation of the images (stimulus-outcomes) or available action (action-outcomes). Monkeys needed to make a lever movement in the direction of the image, or action, which produced the preferential reward. During the choice phase of the task, there was a clear delineation of activity in the ACC for action-outcomes and orbitofrontal cortex (OFC) for stimulus-outcomes, showing the importance of the ACC in action-outcome assessments for optimal decision making. Further, single-cell recordings of ACC neurons have shown specific spikes in activity during the action

component of a delayed attention task, and the related outcome following the action (Hyman et al., 2012).

Single-cell recordings in rodents have also shown that the ERN produced by the ACC occurs specifically when expected actions and expected outcomes do not match (Hyman et al., 2017). In this study rodents were trained to nose poke for reinforcements in three wells, each well had a switch point where it would change from its initial probability of producing a reward to the planned other probability of producing reward. One port was 25/75, where the initial output was 25% and the secondary output was 75%, the second port was 50/50, and the third was 75/25. Scented puffs of air denoted when a nose poke resulted in a reward, and when a nose poke did not result in a reward. Subjects were shown to both understand the differing scents, and to alter behavior to primarily nose poke at the port which was currently producing the heightened reward probability. Single-cell recordings in the ACC showed that there were cells which reliably activated when a nose poke occurred, and when the outcome air puff was delivered. The ERN was seen when incongruent trials had been preceded by multiple correct responses, indicating a prediction error signal as has been seen in previous studies (Warren et al., 2015). These results suggest that the ACC is responsible for guiding changes in action that occur not on the trial-by-trial level, but rather when new strategies (moving to a new port) are needed to optimize reward.

Mesocortical Dopamine

Mesocortical dopamine (DA) has been shown to be crucial for working memory, attentional control when cognitive load is high (Chudasama & Robbins, 2004), and error

monitoring (Bari & Robbins, 2013) in rodents. The rodent mesocortical DA pathway projects from the ventral tegmental area (VTA) to the medial prefrontal cortex (Berger, 1974), where it converges with noradrenergic projections from the locus coeruleus (LC). Medications aimed at impulse control often have general catecholaminergic effects. Stimulant methylphenidate, and non-stimulant atomoxetine, are both used to alleviate the high impulsivity (Roessner et al., 2010). Both have been shown to increase DA and NE in the mPFC at high doses, however, evidence suggests that the NE modulation drives the performance increases in attentional focus seen from these medications (Newman & McGaughy, 2008b). D3 receptor modulation, while not crucial for impulse control, has been shown to improve deficits in error monitoring, leading to increased accuracy from performance adjustment (Bari & Robbins, 2013) and a decrease in misses (Marshall et al., 2019).

Converging evidence suggests that DA projections to the ACC may be crucial for optimal executive performance, by way of integrating attentional and behavior control (Hollerman & Schultz, 1998; Holroyd & Coles, 2002). Increasing medial prefrontal dopamine (ACC and PL) produced increases in the attention phase of a modified 5CSRTT, which included a memory component (Chudasama & Robbins, 2004). When mPFC DA was increased, impulsivity, which has been previously linked to linked the functionality of the PL decreased. Additionally, an increase in dopamine in subjects with low initial accuracy increased response accuracy. Lesions to pregenual ACC have been shown to result in declines in response accuracy (Chudasama et al., 2003; Chudasama & Robbins, 2004). Rodent models utilizing ID/ED shifts have shown that rats sensitized to amphetamine to have difficulty with the EDS (Fletcher, 2005). Similar deficits at this stage of the task have been seen in patients with persisting cognitive schizophrenia symptomology (Jazbec et al., 2007; Pantelis et al., 1999). When a D1 agonist was injected into

the mPFC in amphetamine-sensitized rats, the performance deficits at the EDS were abolished (Fletcher, Tenn, Rizos, Lovic, & Kapur, 2005). Together, the data suggest that optimal levels of dopamine in the ACC may be crucial for response accuracy in a multitude of tasks that require increased cognitive control.

Dopaminergic inputs to the ACC have been predicted to play a major role in the ability to produce an ERN (Holroyd & Coles, 2002). Dopamine neurons have been shown to increase firing rates when outcomes do not match expectations in a positive way, as well as decrease firing rates when outcomes do not match expectations in a negative way (Schultz, 1997). Further, dopamine has been shown to be sensitive to the timing of reward. Single-cell recordings of non-human primates have shown that when a reward is delayed, dopamine neurons will fire at the time point in which the reward was expected to occur, and again when the reward is eventually given (Hollerman & Schultz, 1998). If a reward is predictable, the firing will adjust to match the new timing pattern. If a reward is omitted, firing will increase when the reward was expected, and eventually decline, showing a depression in firing.

It remains underdetermined if dopamine in the ACC is the crucial element of utilizing reinforcement history when expected and actual outcomes are in conflict. To address this gap we tested both male and female rodents with dopaminergic lesions to the ACC in validated tasks of attentional set formation and shifting (Birrell & Brown, 2000), which is sensitive to non-specific lesions to the ACC (Newman & McGaughy, 2011), and sustained attention with varied cognitive distractors (McGaughy & Sarter, 1995).

Assessment of attentional set and weaknesses of The Wisconsin Card Sorting Task

The Wisconsin card sorting task (WCST) has been used as a measure of executive function for over sixty years (Berg, 1948). In the WCST participants are presented with a deck of cards where each card (complex stimulus) contains three dimensions: color, shape, and the number of shapes on the card. An exemplar card for each possible sorting dimension is placed on the table in front of participants. Participants are asked to sort the deck of cards, one card at a time until all cards have been used. With no initial cue to the relevant dimension, participants must utilize trial-by-trial feedback to decide which dimension to sort the cards by; i.e. sort based on color, regardless of number or shape. The relevant stimulus dimension is maintained for an undisclosed, pseudo-randomized amount of time. At which point, the relevant dimension will shift, without the participants' knowledge. At this point, the participant must incorporate the negative feedback to realize that the dimension they were using is no longer correct. At which point, they must shift to a new dimension, uncovered through the trial-by-trial feedback. The task assesses the number of categories achieved, preservative errors, and regressive errors. Failure at the WCST is seen in a low number of categories achieved, accompanied by some form of error. Perseverative errors are errors in which the previously relevant dimension continues to be selected, despite the negative feedback. A regressive error occurs when a participant/subject has made correct sorts following a shift, but does not maintain the correct dimension, and regresses to the previously relevant dimension.

While the WCST has been widely used to explore executive function in humans, the task recruits multiple brain regions, all varying in function (Berman, Ostrem, Randolph, Gold, Goldberg, & Coopola, 1995; Downes et al., 1989; Goldberg et al., 1990; Owen, Roberts, Polkey, Sahakian, & Robbins, 1991). The task requires motivational, attentional, memory, and learning processes to achieve success (Downes et al., 1989). This has been seen in PET data, which has

shown that the WCST recruits a vast network requiring more than just executive function and the prefrontal cortex for success (Berman, Ostrem, Randolph, Gold, Goldberg, Coopola, et al., 1995), strengthening the idea that unilateral failure can be based several independent forms of dysfunction (Berman, Ostrem, Randolph, Gold, Goldberg, Coopola, et al., 1995; Downes et al., 1989; Goldberg et al., 1990). Lesion/regional damage data from functionally distinct brain regions have also shown that the task is not sensitive enough to assess the regionally specific cognitive symptoms of neuropsychiatric disorders. Patients with temporal lobectomy have been tested alongside patients with frontal lobe damage, and early-stage Parkinson's (non-medicated) in the WCST (Canavan et al., 1989). Here, all three groups showed a failure in the WCST, where fewer categories were achieved compared to a younger control group (M = 27.9 years), but the same as an older control group (M = 60.3 years). Showing a clear inability of the task to differentiate failings between functionally distinct regions of the brain. Further, patients with schizophrenia and Huntington's showed no differences in performance on the WCST, although they had markedly different cerebral blood flow during the task (Goldberg et al., 1990).

Another failing of the WCST is that the same compound stimuli (cards) and sorting locations are used across the entire testing session (Berg, 1948). This is important for two reasons, first, utilizing the same stimuli prevents the disentanglement of general stimuli processing failings, from more acute set formation or shift failings (Dias, Robbins, & Roberts, 1996; Dias, Robbins, & Roberts, 1997; Downes et al., 1989; A Owen, A. Roberts, C. Polkey, B. Sahakian, & T. Robbins, 1991). As well as an inability to discern if the concept of a set, or a location was achieved/failed since the location of the stimuli never changes (Downes et al., 1989). Second, In the WCST literature, the Intradimensional set shift is described as a general knowledge that a set is known to exist and is being utilized (Berg, 1948; Downes et al., 1989; A

Owen et al., 1991). However, the WCST never explicitly tests that a set has been formed and can be transferred in a complete changeover design. Having to apply the set to new compound stimuli is never tested in the WCST (Downes et al., 1989; A Owen et al., 1991).

Intradimensional and Extradimensional Set Shifting Tasks

Based on the shortcomings of the WCST to discriminate the sperate cognitive processes involved in the overarching task, the components of the task were disentangled for individual assessment (Downes et al., 1989). Human selective attention literature uses the terminology of Intradimensional and extradimensional shifts to discuss the formation, and flexibility needed to shift an attentional set (Slamecka, 1968). Humans (Buss, 1953), non-human primates (Dias et al., 1997), and rodents (Birrell & Brown, 2000) show selective attention to a stimulus dimension of a compound stimulus when that dimension is repeatedly paired with reinforcement. In these studies, attention to a reinforced stimulus dimension is maintained even when the stimuli are changed. This type of shift is referred to as an Intradimensional shift (IDS). If the rule is changed, and a previously irrelevant dimension is now paired with reward, and extradimensional shift (EDS) has occurred. The use of an attentional set can be seen in a better IDS compared to EDS performance (Birrell & Brown, 2000; Buss, 1953; Dias et al., 1997; Downes et al., 1989; Slamecka, 1968).

In an Intradimensional extradimensional set shifting task (ID/ED task) the core components of the WCST are elongated and can be analyzed as the distinct processes they are (Downes et al., 1989). The hallmark of the WCST is an extradimensional shift (EDS), this is

when the correct stimulus dimension changes and the set must be shifted. All shifts in the WCST are akin to the EDS. Not explicitly tested in the WCST are Intradimensional shifts, the difference in performance between the two being the hallmark of set formation (Buss, 1953). In the WCST the location in which the cards are to be sorted based on stimulus dimension never change, this can lead to decisions being made based on a memory of a location as opposed to the actual formation/application of an attentional set (Downes et al., 1989). In the ID/ED task pairs of compound stimuli are shown, and the location (left or right) of the rewarded stimulus is pseudo-randomized to prevent location-based responses (Birrell & Brown, 2000; Dias et al., 1997; A. Owen, A. Roberts, C. Polkey, B. Sahakian, & T. Robbins, 1991).

The ID/ED tasks are sensitive to functionally distinct regions (Birrell & Brown, 2000; Newman & McGaughy, 2011; Tait & Brown, 2007), as well as to neurochemical assessment (McGaugh y et al., 2008; Newman & McGaughy, 2008a), allowing for fine-tuned assessment of the function of the regions of the prefrontal cortex, and associated neuropsychiatric disorders. When patients who had undergone temporal lobectomy for epilepsy were tested in an ID/ED task, also compared to young and older control groups, they showed only an increased latency on the EDS. Showing, no comparable differences in set formation, shift, or reversal learning compared to young controls (Owen et al., 1991). Patients with frontal lesions, and older controls much like in Canavan 1989, showed impairments at the EDS stage of the task. however, the ID/ED task revealed a marked difference from the WCST data, showing that there were no IDS differences between any of the groups (Owen et al., 1991). Patients with dopaminergic related pathologies such as schizophrenia (Dolan et al., 1995), and Parkinson's (PD)(Lotharius & Brundin, 2002) have shown specific deficits in the attentional set shifting task which are not examinable in the WCST (Downes et al., 1989). Patients with schizophrenia have shown

difficulty at both the IDS and the EDS (Pantelis et al., 1999). Patients with Parkinson's have been shown to have specific deficits in the EDS (Owen et al., 1993).

Lesions to rodent medial prefrontal cortex produced deficits in the EDS stage of the task (Birrell & Brown, 2000). Healthy adolescent rats also show severe cognitive rigidity, with higher than average trials to complete the EDS stage accompanied by significant increases in perseverative errors (Newman & McGaughy, 2011a). Ibotenic acid lesions (IBO-LX) to the anterior cingulate cortex have been shown to increase susceptibility to distraction when previously reinforced attributes of a compound stimulus are introduced in a complex stimulus, but no difficulty with set formation or shifting (Newman & McGaughy, 2011), indicating a specific function for the ACC which is not examinable using the WCST. Additionally, when healthy adolescent rodents and adults were compared in the ASST, adolescents were shown to have increased trials to criterion at the CD stage, but also formed an attentional set (Newman & McGaughy, 2011a). One of the anatomical differences which are expected to underlie human adolescent susceptibility to distracting stimuli is an underdeveloped ACC (Casey & Jones, 2010). The behavioral similarities of IBO-LX subjects (Newman & McGaughy, 2011) and healthy adolescents(Newman & McGaughy, 2011a) shows support for this idea.

The Sustained Attention Task

Sustained attention, also referred to as vigilance, is a maintained state of readiness to detect and subsequently respond to unpredictable target stimuli (Parasuraman & Davies, 1976). The classic example of vigilance decrement was seen when Mackworth (1948) noted that radar operators begin to detect fewer targets after 30min of time spent at the scanner. This idea of a

decrease in the ability to detect signals over time is a key feature of vigilance decrements. A vigilance decrement occurs from a decrease in perceptual sensitivity when targets and non-targets are discriminated from memory in rapid succession (successive signals) (McGaughy et al., 1996; McGaughy & Sarter, 1995; Parasuraman & Davies, 1976). If memory load is not increased by the task (simultaneous signals), vigilance decrements are based on changes in response criteria (Parasuraman, 1979). Parasuraman's taxonomy of vigilance tasks comprises of four main dimensions: signal type, event rate, modality, and source of complexity. To accurately test vigilance decrements that are based on decreases in perceptual sensitivity five main themes emerge. Successive presentations of signal and non-signal trials result in a heightened memory load (Parasuraman, 1979). High event rates are necessary for conjunction with successive presentations to elicit a vigilance decrement based on a decrease of signal detection (Parasuraman, 1979). The ability to detect less salient (shorter in presentation duration) signals decreases more over time compared to more salient (longer in presentation duration) signals (Parasuraman & Davies, 1976). More complex response rules require more resources and will increase sensitivity to manipulations which also tax resources (Parasuraman, 1979). And finally, that the temporal unpredictability of a signal or non-signal event is crucial to assessing a vigilance decrement (Parasuraman, 1979).

McGaughy and Sarter (1995) developed an operant box task for rodents which is used to assess vigilance decrements in a baseline task, as well as in task variants which manipulate the load on varying cognitive processes which has since been translated for use in humans, both healthy and with neuropsychiatric pathology (Demeter et al., 2013). In the baseline task, animals must discriminate between temporally unpredictable signal (light presentation) and non-signal (no light presentation) trials, where the signal trials are embedded in a dynamic stimulus range.

The task addresses the five Parasuraman components of a vigilance decrement task as follows: signal and non-signal trials are presented in pseudo-randomly in rapid succession, where memory is necessary and taxed for correct responses. Event rates are maintained both unpredictably, and in rapid succession with an inter-trial interval of 12±3 seconds. The task includes a dynamic stimulus range where targets are presented for 500msec, 100msec, or 25msec. Importantly, the SAT consistently demonstrates signal-dependent performance, where subjects' accuracy is highest for the 500msec signals, and lowest for the 25ms signals (McGaughy & Sarter, 1995; Newman & McGaughy, 2008b, 2011). Finally, the task has a complex response rule which requires more than a simple response. In this task, a signal presentation requires a left lever selection (hit), a non-signal trial requires a right lever selection (correct rejection). Misses occur when a signal trial occurs and an animal makes a right lever selection, and a false alarm occurs when an animal reports having seen a signal (left lever) when none was presented (McGaughy & Sarter, 1995).

The neurochemical circuit involved in the detection of signals in the sustained attention task has been well defined (Gill, Sarter, & Givens, 2000; McGaughy & Sarter, 1995, 1999; Newman & McGaughy, 2008b; Sarter, Givens, & Bruno, 2001). The ability to detect signals over time on task is dependent on a functional acetylcholine system, where following a cholinergic lesion of the basal forebrain, subjects showed a decrease in accuracy for signal trials over time on task, which did not improve over repeated sessions (McGaughy et al., 1996). In the SAT, top-down control is needed when the cognitive load is high (increased time on task, distractors, drugs, etc.), and has been linked to the cholinergic frontoparietal networks in humans, with important nodes in the ACC, ventromedial prefrontal cortex (VmPFC), ventrolateral prefrontal cortex (VLPFC), and dorsolateral prefrontal cortex (DLPFC) (Peterson &

Posner, 2012; Sarter, Gehring, & Kozak, 2005; Sarter et al., 2001). Bottom-up control is seen primarily in the baseline task, which has been hypothesized to come from communication between the PPC and the PFC (Gill et al., 2000; Sarter et al., 2001). Following cholinergic specific lesions to the PFC in rodents, subjects were more susceptible to novel, cross-modal distractors than sham-LX subjects and showed both bottom-up and top-down processing deficits (Newman & McGaughy, 2008b). The sustained attention task allows for a translational assessment of vigilance, the impact of novel distractors and the subsequent need for top-down control, the ability to filter novel, cross-modal stimuli, and the ability to update reinforcement contingencies. Modifications to the SAT where reinforcement timing is manipulated have yet to be tested following lesions to the ACC, and may be related to dopamine in the ACC.

The Hierarchical reinforcement learning theory of anterior cingulate cortex (HRL-ACC)

The Hierarchical reinforcement learning framework of anterior cingulate cortex (HRL-ACC) proposes a role for not only the ACC in modulating behavior but specifically, the role of dopaminergic input to the ACC's role in initiating cognitive control mechanisms (Holroyd & Umemoto, 2016). This framework uses the actor-critic architecture from reinforcement learning theory (RL). In basic RL, actors initiate actions and the critic monitors reward information. Both the actor and the critic generate their respective components of the learning system (policy and value function) from experience, where the critic relays reward prediction errors to the actor (positive, the first time a reward is encountered; negative, when no reward is encountered and expected). Together the actor and critic create a model that can be used to navigate relatively simple behavioral tasks (Holroyd & Yeung, 2012). When the word or task becomes more

complex, Hierarchical reinforcement learning algorithms (HRL) can be used to model these more intricate mechanisms for task success. In HRL, higher-level behavioral plans (options) are incorporated into the model. Options are sequences of actions that are aimed at achieving sub-goals, which in turn will lead to the achievement of the overall goal.

The HRL-ACC framework posits that the ACC acts as an agent in the actor-critic model and is responsible for learning the value of tasks, selecting task sets based on their learned values, and motivating the task execution (option selection) by recruiting the dorsolateral prefrontal cortex (DLPFC) and dorsal striatum (DS) (actor). In this framework, the orbitofrontal cortex (OFC), and the dopaminergic system are responsible for monitoring reward feedback and sending this information to the agent and actor (critic). These three units, agent, actor, critic, are responsible for the successful completion of tasks that employ complex decisions and tax attentional load. Tasks that require complex decisions can be seen when multiple attributes of a stimulus may be predictive of reinforcement. To ascertain which aspect of a stimulus should be attended and which should be filtered (option selection) subjects must utilize reinforcement feedback to generate errors.

Dopaminergic projections to the ACC result in the reward positivity error (RPE) (also known as feedback error-related negativity) component of an ERP (Holroyd & Yeung, 2012; Warren et al., 2015). The RPE increases when a reward is better than expected and ceases when a reward is worse than expected. The latter has been related to the error-related negativity (ERN) component of an ERP which occurs in the ACC following the commission of an error (Gehring, B., Coles, Meyer, & Donchin, 1993; Hyman et al., 2017; Laurens et al., 2003; Scheffers et al., 1996). This dopaminergic signaling in the ACC is hypothesized to be utilized to adapt behavior to make the most advantageous option selection for maintaining reinforcement on a global scale

(Holroyd & Umemoto, 2016). The framework states that in the absence of ACC control over action production or motivational supervision, behaviors should become slower, and less accurate/more susceptible to distractions.

The actor-critic ability to make trial-by-trial modifications without the ACC coheres with the IBO-LX and DA-LX subjects abilities to make conditional discriminations in the exemplar and SD stages of the attentional set shifting task, as well as subjects ability to learn the complex response rules in the sustained attention task (Newman & McGaughy, 2011). Further, the HRL-ACC framework suggests that ACC dysfunction may result in an inability to have a positive value for the task as a whole, decreasing attention to task-specific demands when situations become complex (introduction of distractors, extended time on task, etc.) (Holroyd & Umemoto, 2016). While the decreased performance in the attentional set shifting task in the face of complex stimuli supports this, the lack of detrimental performance in the face of novel distractors in the sustained attention task indicates a more specific form of distractibility when the ACC is dysfunctional (Newman & McGaughy, 2011). Subjects showed an inability to ignore attributes of a complex stimulus that had a prior reinforcement history. In the HRL-ACC framework, this could be seen as an inability to engage in option selection, where prior reinforcement feedback, as well as current reinforcement feedback, was being utilized by the actor, increasing regressive errors, where subjects were slow to learn the new alternative exemplar in the reinforcement reversals, but did not show an adherence to the exemplar explicitly reinforced in the previous stage of the task.

Dopamine as a Stability Mechanism

While the HRL-ACC framework is well-positioned to interrogate the function of dopamine in the ACC, some gaps remain, which may be filled in by the theory of dopamine in the ACC as a stability mechanism (Chudasama & Robbins, 2004; Robbins, 2005). The hypothesis is that dopamine in the ACC is necessary for the integration of stimuli and the increase in behavioral control needed when situations are complex. Non-specific damage to the ACC in rodents has produced deficits in attention, but not impulsivity or working memory in a 5-CSRTT (Chudasama et al., 2003). Dopaminergic modulation of the mPFC (both ACC and PL) showed a specific increase in attention, but not working memory in a modified 5-CSRTT with a mnemonic component (Chudasama & Robbins, 2004). Taken together, the data indicates a role for dopamine in promoting the stability of incoming stimuli representations and decreasing the susceptibility to distraction.

In patients with low dopamine synthesis, D_2 receptor stimulation has been shown to improve task-switching ability (van Holstein et al., 2011). Task switching requires the integration of stimuli and feedback (from reinforcement, punishment, or errors) to determine the relevant stimuli or dimension and employ the necessary cognitive control to adjust to the new task set (Sakai, 2008). Dopamine may not be the key factor in forming a task set, however, it is hypothesized that dopamine is necessary to maintain the task set and prevent distraction (Robbins, 2005). The ACC has been shown to play a crucial role in maintaining task sets, meaning it is crucial for holding the set of rules which guide the optimal decision making (Sakai, 2008). Dopamine in the DLPFC in humans is thought to play a role in dampening distractors, while norepinephrine is thought to increase the signal salience of target stimuli (Arnsten, 2011). This dual role for the increase in catecholamines in adjusting the signal to noise ratio has been seen in the beneficial use of methylphenidate in modulating performance in tasks which require

cognitive control (Marshall et al., 2019; Roessner et al., 2010; Sonuga-Barke et al., 2007). In conjunction with the HRL-ACC framework, dopamine in the ACC may be crucial for selecting and maintaining the task set, and relaying that information to the DLPFC, which in turn adjusts the signal to noise ratio accordingly. If there is no stable representation of the task set (current rules/goals) from the ACC from a lack of dopaminergic signaling, other rules or goals (prior reinforcement history) may provide a source of distraction.

Current Study

Dopaminergic lesions to the ACC in rodents should allow for the maintained communication between the actor and critic, as in seen in basic RL (Holroyd & Yeung, 2012). This should facilitate RL for the conditional discriminations when stimuli are simple in the attentional set shifting task as well as the ability to learn the reinforcement rule in the sustained attention task (Newman & McGaughy, 2011). When faced with complex stimuli, dopaminergic lesions to the ACC should produce increased susceptibility to distraction, as seen with previous IBO-LX to the ACC, specifically when complex stimuli contain stimulus dimensions with a prior reinforcement history (Newman & McGaughy, 2011). Further, this distractibility has only been seen when the varying irrelevant dimension is changed, and performance is not impaired when the consistent dimension is altered. This specific distractibility would cohere with the HRL-ACC framework, and the specific role of dopamine in integrating incoming stimuli and error feedback to generate the overall dimension selection and implement the necessary recruitment of the actors (Chudasama et al., 2003; Holroyd & Umemoto, 2016; Holroyd & Yeung, 2012; Robbins, 2005). Distractibility to novel distractors has been linked to cholinergic deafferentation of the

prelimbic cortex (Newman & McGaughy, 2008b), and has not been shown with damage to the ACC (Newman & McGaughy, 2011). As such, increased susceptibility to distraction from novel distractors in the sustained attention task is not predicted from dopamine lesions to the ACC. An inability to produce a reward prediction error should diminish the ability of the ACC to select an appropriate option (stimulus dimension) in the face of complex stimuli and may also dampen the signal needed to elicit increased cognitive control in order to maintain attention on the relevant stimulus dimensions.

The attentional set shifting task and the sustained attention task have been previously assessed in both male and female rodents. In the attentional set shifting task no sex differences have been shown in healthy adult controls or subjects with exposure to anesthesia (Murphy et al., 2016). Further, unpublished data from the McGaughy lab has shown that male and female adolescent rodents show the same deficits at the CD and ED stages of the task (McGaughy, unpublished). Female and male rodents have also been assessed in the sustained attention task. Male and Female rodents showed no difference in the ability to acquire criterion accuracy in the sustained attention task (McGaughy & Sarter, 1999; Murphy et al., 2016). Female rodents show no change in performance in the phases of the estrous cycle (McGaughy & Sarter, 1999). Following cholinergic lesions, to the basal forebrain, both males and females showed deficits in signal detection over time on task (McGaughy et al., 1996; McGaughy & Sarter, 1999). In the present study, no sex differences are expected in the attentional set shifting task or the sustained attention task.

CHAPTER II:

THE IMPACT OF DOPMINERGIC DEAFFERENTATION OF THE ANTERIOR CINGULATE CORTEX IN AN ATTENTIONAL SET SHIFTING TASK

Converging evidence from single-cell recording (Hyman et al., 2017; Hyman et al., 2012; Warren et al., 2015), lesion (Chudasama et al., 2003; Kennerley et al., 2006; Newman & McGaughy, 2011; Passetti et al., 2002), and pharmacology studies (Chudasama & Robbins, 2004) using rodent models suggest a role for the ACC in incorporating error feedback to determine relevant and irrelevant dimensions of a stimulus. Specifically, the HRL-ACC framework suggests that dopamine in the ACC is crucial for generating the error feedback and integrating this information with incoming stimuli to implement task sets, which are used to guide actions when the need for attentional control is high (Holroyd & Umemoto, 2016). Previous studies in rodents have shown that loss of the ACC, but not PL/IL or OFC decreases the ability to generate a temporal action plan when high attentional demands are present (Chudasama et al., 2003; Passetti et al., 2002). Subjects have also been shown to have an increased susceptibility to distraction when stimuli were complex, but not when novel distractors were present, indicating a role of previous reinforcement history in facilitating distractibility (Newman & McGaughy, 2011).

Subjects with ACC lesions in both the 5CSRTT (Chudasama et al., 2003; Passetti et al., 2002) and the ASST (Newman & McGaughy, 2011) showed no increase in perseverative errors, unlike lesions to IL/PL (Chudasama et al., 2003; Newman & McGaughy, 2008a). The lack of

perseverative errors in either task suggest that subjects with lesions to the ACC do not simply continue to respond to the previously reinforced task element, be it the nose port, or stimulus dimension; rather, subjects in both tasks show disorganized responses to dimensions which have at some point in testing previously elicited a response. Non-specific lesions to the ACC did not produce an increased susceptibility to all distraction, as deficits were seen at other stages of the ASST where no novel stimuli were presented, and the presence of novel stimuli in a sustained attention task did not produce a decrease in accuracy compared to subjects with sham lesions (Newman & McGaughy, 2011). Dopamine agonists have been shown to increase accuracy in the 5CSRTT in poor-performing rodents (Chudasama & Robbins, 2004), possibly indicating a need for optimal levels of dopamine in the ACC to facilitate the use of global task sets.

Dopaminergic projections to the ACC have been hypothesized to modulate the integration of error signals following reinforcement feedback (Holroyd & Coles, 2002; Holroyd & Umemoto, 2016; Holroyd & Yeung, 2012). The signals generated by error and feedback have been shown to depend on the functionality of the ACC in rodent models (Hyman et al., 2017; Hyman et al., 2012; Warren et al., 2015). The HRL-ACC framework postulates that dopamine in the ACC integrates the error signals and the incoming stimuli. This integration is used by the ACC to determine an appropriate action plan and recruits the dorsolateral prefrontal cortex and dorsal striatum to carry it out (Holroyd & Umemoto, 2016). Dopamine has also been posited to serve as a stability mechanism for these action plans, where without it, they become susceptible to distraction (Robbins, 2005). Previous non-specific lesion studies of the ACC have shown an increased susceptibility to distraction from previously reinforced attributes of a complex stimuli, but not novel stimuli (Newman & McGaughy, 2011). The specific role of dopamine dysfunction

in the ACC in producing that susceptibility to distraction from reinforcement history remains unexaminable without a dopamine specific lesion to the ACC.

We hypothesized that dopaminergic deafferentation of the ACC would be sufficient to produce deficits in the ability to incorporate error information based on reinforcement feedback only in situations of high attentional load in the ASST. This inability to overcome a previous reinforcement history would indicate that dopamine in the ACC is only necessary when situations require the integration of reinforcement feedback and incoming complex stimuli. When stimuli are simple, no action/task selection is needed, as trial-by-trial feedback can be used to accomplish the goal (Holroyd & Umemoto, 2016). However, in situations of increased attentional load, subjects may require the ACC to specify which dimension of a complex stimulus is task-relevant, and which is irrelevant. Without dopamine, this selection is vulnerable to prior reinforcement history, as has been seen in the random port choice in the 5CSRTT, and increased susceptibility to distraction in the ASST following non-specific lesions to the ACC. As the impact of dopamine in unexaminable in non-specific lesion studies, the present study assessed the cognitive deficits following dopaminergic deafferentation of the ACC.

In the present study, an anti-dopamine transporter saporin (anti-DAT; Advanced Targeting Systems, San Diego, CA) was used to deplete dopamine in the ACC of 18 rodents (10 male, 8 female). In the control subjects (18 rodents; 10 male, 8 female), the anti-DAT saporin vehicle Dulbecco's saline was injected into the ACC, allowing for a surgical procedure without cellular damage. DAT is a sodium-dependent reuptake carrier for dopamine. It terminates dopamine transmission by returning dopamine to the presynaptic cell. The anti-DAT saporin binds to DAT and enter dopamine positive cells, at which point the saporin can cause cell death via apoptosis (Wiley, Harrison, Level, & Lappi, 2003) This toxin is not only selective to

dopamine, but is regionally specific, and does not impact dopaminergic cells outside of the targeted area. The impact of this dopaminergic deafferentation of the ACC on specific distractibility to previously reinforced attributes of complex stimuli, as previously seen in (Newman & McGaughy, 2011) was assessed in an attentional set shifting task.

Research Design and Methods

Procedure

Animals were pair housed (separated by sex) and maintained *ad libitum* access to food and water for five days. During this time, animals were gently handled and acclimated to the surgeon, testers, transport boxes, and surroundings. On the sixth day, animals were weighed, moved to separated housing units, and were restricted to 3 pellets <23h prior to surgery. Animals were randomly assigned to lesion condition, 10 male DA-Lx, 10 male Sham-Lx, 8 F DA-Lx, and 8 F Sham-Lx.

On the 18th day after arrival animals began initial training in the operant boxes and were maintained on a food restriction of 16g for males and 14g for females. Regardless of success, animals would run a minimum of four initial training days in the operant boxes, to ensure that behavioral testing would not begin until a full two weeks after surgery had been performed. Following initial training rats begin testing in the attentional set shifting task (ASST). The ASST was conducted over two-three consecutive days. All animals were tested between 12:00pm and 4:00pm, with all testing sessions occurring at the same time for each animal. Session 1 consisted of training to dig and four sets of discriminations: exemplar training (3), and the simple

discrimination. Session 2 consisted of seven sets of discriminations: the compound discrimination stage though learned irrelevance. If an animal emitted eight errors of omission in a row, testing concluded for that animal for the day and was resumed 24h later. Following completion of the ASST all animals were assessed in a sustained attention task, i.e. experiment 2 (See Chapter 3).

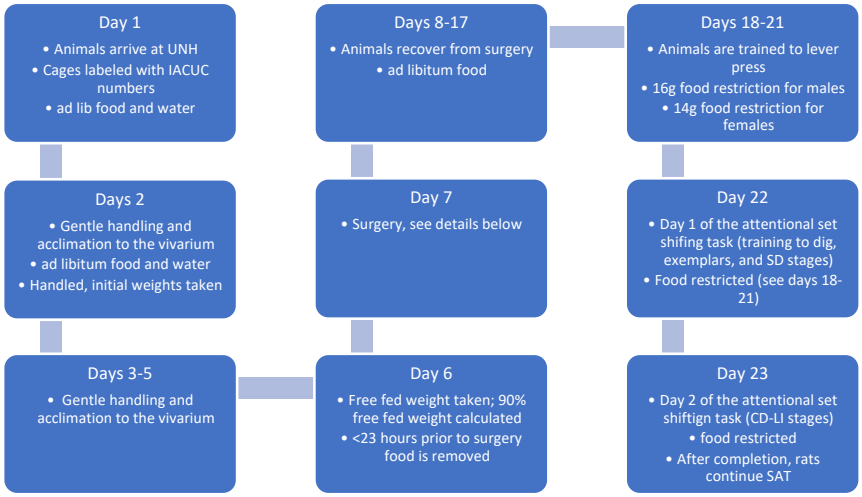

Figure 2.1 timeline of the procedure from animal arrival to the end of behavioral assessment in the ASST. Timeline continues for experiment two in chapter 3 on page 45.

Surgery

Rats were anesthetized with a cocktail of ketamine (85mg/kg) and xylazine (8.5mg/kg). Once no pain response was elicited from a toe pinch, rodents were shaved (10 razer, Oster, Newell Brands, Atlanta, GA) from nose to ears and placed onto a stereotaxic frame (Stoelting, Wood Dale, IL) secured with atraumatic ear bars (Stoelting, Wood Dale, IL). The upper teeth were placed into the tooth bar (set at +3.3) and secured with the nose clamp. Eye Soothe PM Ointment (Rite Aid, Camp Hill, PA) was applied to the eyes to prevent drying. The coordinates

for the ACC were as follows : AP: +2.7 ML: ±0.6 DV: -2.4; AP: +2.2 ML: ±0.6 DV: -2.2 (Chudasama et al., 2003). The ACC was injected with 0.2 µg/µL Anti-DAT saporin or it's vehicle, Dulbecco's saline. Each of the four 0.2 µl/site injections occurred at a rate of 125nL/min to prevent unwanted diffusion of toxin and cell damage. All injections occurred with the bevel facing the midline, and with the needle remaining at the injection site for four minutes prior to, and after injection, to prevent diffusion outside of the target area. Following surgery antibiotic ointment was applied to the sutures, and the rats were observed and heated (soft heat, perfect fit, Charlotte, NC) until fully mobile and capable of ingesting a meloxicam tablet (Bacon Flavor 0.5mg, Bio Serv, Flemington, NJ). After surgery rats received Neosporin pain+ (Johnson & Johnson, New Brunswick, NJ) twice daily on their sutures, as well as a daily meloxicam tablet (Bio Serv, Flemington, NJ). Weights were recorded daily for five days following surgery to ensure weight gain, indicating positive recovery.

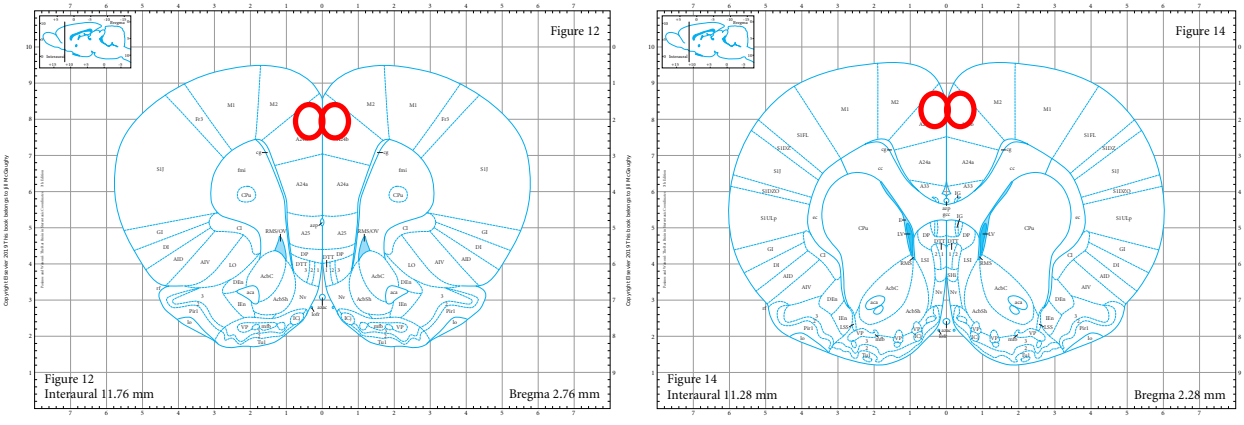

Figure 2.2 locations of the four injection sites into ACC from paxinos and watson 7th edition of *the rat atlas in stereotaxic coordinates*. Left: rostral injection site +2.7 AP, ±0.6 ML, -2.2 DV. Right: caudal injection site +2.2 AP, ±0.6 ML, -2.7 DV.

Behavior

Apparatus and Materials

Operant chambers (Med Associates, St. Albans, VT) with two retractable levers, three panel lights (2.8 W), a back panel houselight (2.8 W), a sonalert tone generator, factory set at 2900Hz, and a 45 mg food pellet dispenser. The retractable levers, panel lights, tone generator, and pellet dispenser were all located on the same wall of the operant chamber. The houselight was on the wall opposite, with the open end of the light cover aimed at the ceiling. Data collected from the Med-PC IV 4.25 software (Med Associates, St. Albans, VT) was maintained on the desktop running the operant boxes, both with Windows XP (Microsoft, Redmond, WA). Recorded was signal duration, intertrial interval duration, lever selection, errors of omission, and food pellet (Bio Serv, Flemington, NJ) acquisition.

All testing sessions were conducted in a plastic testing box (L X W X H; 53 X 41 X 29cm) with an opaque plastic divider (L x W x D; 28.5 X 40.5 X 0.3 cm). Rats were trained to dig in terracotta pots with a height of 10cm and an internal diameter of 10.2cm. Pots were filled with clear wax and gravel ¾ of the way to weight pots. Pots were secured to the testing box floor with Velcro (Velcro Industries N.V., United Kingdom) to decrease the likelihood rats would tip the pot over. The pots served as the complex stimuli in the task. Pots could vary on three dimensions: Odor (diluted essential oils 1:100 in canola oil), Digging media, and texture (figure 2.3). On the simple discrimination, test stimuli differed on only one of these dimensions, all other discriminations used complex stimuli differing on two dimensions with the third dimension identical across testing pairs.

Training

Shaping to lever press: Rats were trained in operant boxes (Med Associates, St. Albans, VT) to lever press for food reward (45mg pellets, BioServ, Flemington, NJ). In this initial shaping, both levers extend into the box. The rat must depress either lever to receive food reward. To prevent the formation of a side bias, levers become "locked out" after 5 consecutive depresses of one lever (left or right), where the rat must depress the other lever to receive reward. Should no lever be depressed within 4min a pellet will be dispensed. Sessions run for 45min or until the maximum, 120 rewards, is reached. To be eligible to progress to training, rats must make at least 25 lever presses on each lever and receive over 50 rewards.

Training to Dig: Rats were trained to dig for reward in terra cotta pots filled with unscented manila folder. Digging was defined as displacing the digging media with a nose or a forepaw. 45mg pellets (Bio Serv, Flemington, NJ) are placed for three trials on top of the manila folder, partially buried in the manila folder for six trials, and finally ten trials with the pellets fully buried. Any trial in which a rat does not dig within the allotted 90seconds, the trial is recorded as an error of omission, and the trial repeated until the required number of digs has been met (3, 6, and 10 respectively). If an subject made eight omissions in a row, they were returned to their homecage for thirty minutes, at which time they would begin with the stage (top, partially buried, or fully buried) of training to dig they emitted eight omissions in. Should another eight omissions be emitted in a row, subjects were done testing for the day. Subjects were retuned to the homecage, and their food restricted was tightened (2g less).

Discovery Trials: For every set of discriminations following training to dig, testing sessions begin with four discovery trials. Discovery trials differ from standard trials in two ways. First,

during discovery trials, should an incorrect choice be made, subjects are allowed to explore the correct pot. Second, discovery trials have a 90 second limited hold as opposed to the 60 second limited hold seen in all other trials. Should no response be made in the 90 seconds, the trial is marked as an error of omission, and the subsequent trial runs as a discovery trial until 4 responses have been made.

Criterion Performance: In all trials the location of the baited pot (right or left) is pseudorandomized. All pots are crush reinforced with food so animals cannot base sections on pellet scent. For all trials, response accuracy (correct and incorrect) and response latency are recorded. Rats must emit six consecutive correct responses to proceed to the subsequent stage of the task.

Exemplar Training: Following training to dig, animals are introduced to the dimensions of the future complex stimuli (odor, texture, digging media). This is conducted as a set of simple discriminations where the pots only differ on one dimension. For example, in a texture exemplar the two pots presented contain no odor, and a manila digging medium, but one pot is covered in fur, and the other pot is covered in reverse fur. Utilizing the same material, e.g. fur and reverse fur controls for scent cues. In digging media-based sequences, all materials are set up such that all discriminations are matched for brightness. The order in which exemplars are conducted is dependent on the shift that is occurring.

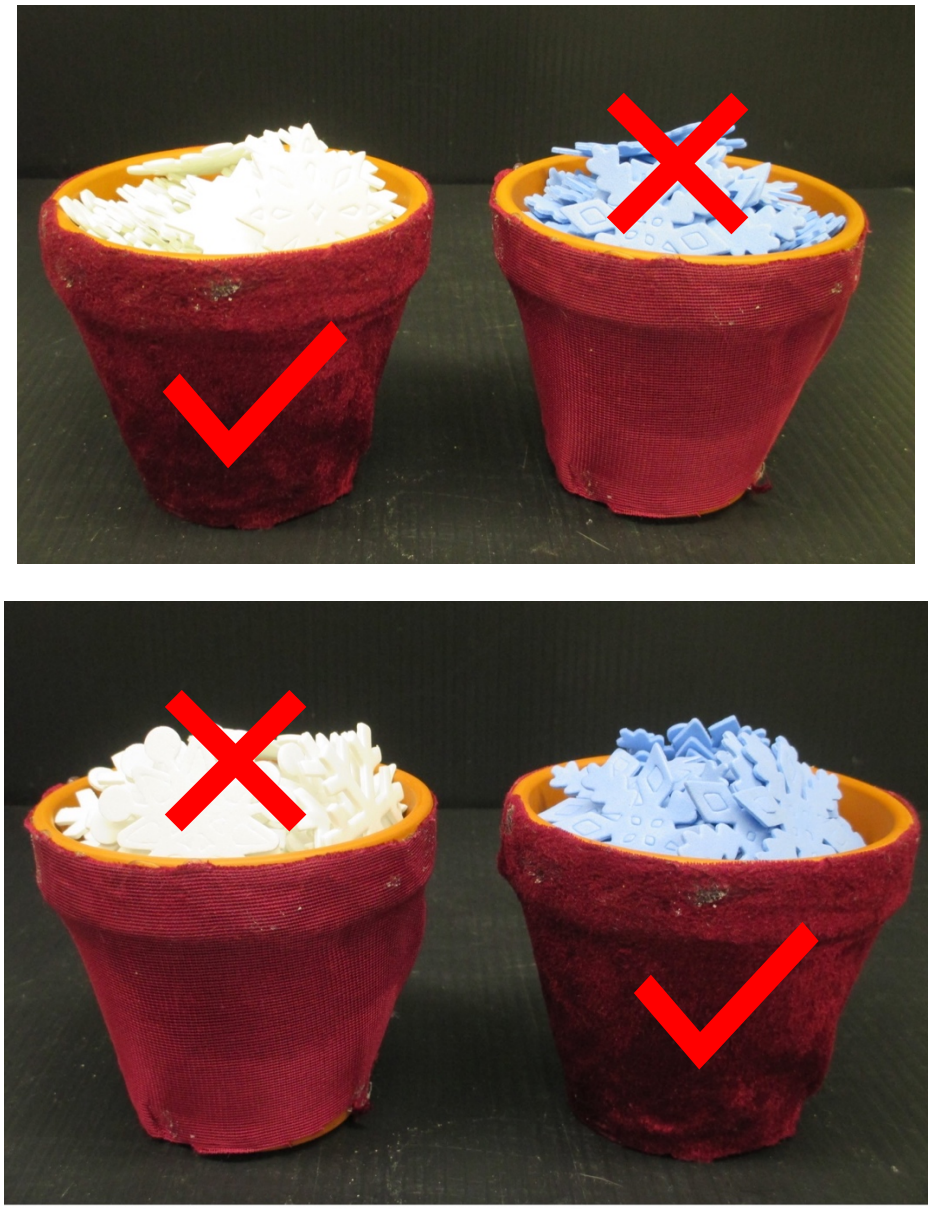

Figure 2.3: Example of the compound stimuli pairs presented in the attentional set shifting task. Here the dimension being reinforced is texture, and in both pairs, the pot covered in velour is baited. In pair 1 (top), velour is presented with light foam shapes, and in pair 2 (bottom), velour is presented with dark foam shapes. Subjects must maintain selection of velour, regardless of the digging media.

Stages

Simple discrimination: In the simple discrimination (SD) stage of the task, rats are presented two pots which only differ on one dimension. Here, one of the pots is consistently baited and the location of the pot (right or left) for each trial is pseudo-randomized. Animals are rewarded for attending to the stimulus attribute previously seen in the final exemplar, in the example shown in table 1, animals will be presented with an velour pot, which is baited, and a reverse velour pot, which is not baited.

Compound discrimination: In the compound discrimination (CD), subjects are tested with complex stimuli, which contain all three stimulus dimensions. Here, the animal needs to continue to attend to the stimulus previously reinforced in the SD, ignoring the new stimulus dimension. Table 1 shows a stage by stage breakdown of the stimuli used in one of the possible sequences (texture to digging media), in this example, the relevant stimuli dimension is texture. In the CD the subject receives reward for digging in the velour textured pot, regardless of if the pot contains light, or dark foam shapes. In all complex stimuli the never relevant dimension is unified across all stimuli, In the example given in table 1, the never relevant dimension is odor, and so all pot pairs used throughout the task stages will have the same odor.

Compound Reversal: In the first reinforcement reversal (CDR), the same stimuli are utilized as the previous stage in the task. What is assessed is the ability to attend to the alternative stimulus

in the pair. In the example given in figure 2.3, velour covered pots were baited in the CD, in the reinforcement reversal, the reverse velour covered pots will now be baited. Animals must select reverse velour, regardless of if the pot contains dark or light foam shapes. Reinforcement reversals are interleaved throughout testing, where the alternate stimulus in the pair needs to be selected to receive reward.

Intradimensional Shift/reinforcement reversal: In the Intradimensional shift (IDS) stage of the task, the animal is required to continue to attend to the same stimulus dimension as the previous stage (texture). Here, the complex stimuli are changed in a total changeover design. If the animal has formed an attentional set, this stage of the task should require fewer trials to criterion compared to the CD stage of the task (Birrell & Brown, 2000; Newman & McGaughy, 2008a, 2011; A. Owen et al., 1991).

Extradimensional Shift/Reinforcement reversal: After successful completion of the Intradimensional reversal (IDR), animals are progressed to the extradimensional shift (EDS) stage of the task. Here, a new set of stimuli are presented, and animals must learn to attend to a previously irrelevant stimulus dimension, shifting the attentional set (texture to digging media). If an attentional set has been formed, the EDS should require more trials to criterion compared to the IDS (Birrell & Brown, 2000; Newman & McGaughy, 2008a, 2011, 2011a; A. Owen et al., 1991).

The Test of Learned Irrelevance: After successful completion of the EDR, animals are progressed to the final stage of the task, the test of learned irrelevance (LI). Here, the never

relevant stimulus dimension is changed. Animals must continue to attend to the previously rewarded stimulus dimension and pair from the EDR, ignoring the complete change of the never relevant stimulus dimension.

Table 1:

Examples of testing pairs over stages in the ASST where bolded stimuli represent the stimuli dimensions that should be attended at each stage of the task.

Task	Testing Pair 1	Testing Pair 2	Non Relevant Attribute
SD	**Velour** vs. Reverse Velour		
CD	**Velour**/Light shapes vs. Rev. Velour/Dark shapes	**Velour**/Dark shapes vs. Rev. Velour/Light shapes	Cinnamon
CDR	**Rev. Velour**/Light Shapes vs. Velour/Dark Shapes	**Rev. Velour**/Dark Shapes vs. Velour/Light Shapes	Cinnamon
IDS	**Terrycloth**/Metallic Beads vs. Rev. Terry/Non-Metallic Beads	**Terrycloth**/Non-Metallic Beads vs. Rev. Terry/Metallic Beads	Gardenia
IDR	**Rev. Terry**/Metallic Beads vs. Terrycloth/Non-Metallic Bead	**Rev. Terry**/Non-Metallic Beads vs. Terrycloth/Metallic Beads	Gardenia
EDS	**Gold Buttons**/Corduroy vs. Dark Buttons/Rev. Corduroy	**Gold Buttons**/Rev. Corduroy vs. Dark Buttons/Corduroy	Rose
EDR	**Dark Buttons**/Corduroy vs. Gold Buttons/Rev. Corduroy	**Dark Buttons**/Rev. Corduroy vs. Gold Buttons/Corduroy	Rose
LI	**Dark Buttons**/Corduroy vs. Gold Buttons/Rev. Corduroy	**Dark Buttons**/Rev. Corduroy vs. Gold Buttons/Corduroy	Lilac

Statistical Analysis

All statistical analyses were performed with SPSS v. 26.0 (SPSS, Chicago, IL). Previous research has shown that different stages of the ASST require functionally distinct areas of the prefrontal cortex (Birrell & Brown, 2000; McGaugh y et al., 2008; Newman & McGaughy, 2011; Tait & Brown, 2007). Because these regions assess varying cognitive domains, several

separate mixed factor analyses of variance (ANOVAs) were conducted to distinguish performance in these domains. In the case of a violation of sphericity, the degrees of freedom were corrected using the Huynh-Feldt correction. Epsilon (ε) values not equal to 1 are reported below. A Bonferroni correction was used to modify alpha levels in the case of multiple post-hoc comparisons. The data was screened for sequence and tester effects, none were found. Three animals were removed from the analysis (two males with DA-LX and one female with DA-LX) due to insufficient histological assessment. Data are presented in the chronological order, they were collected. A full description of histological results is available in Chapter IV.

In order to assess the initial acquisition of the task the first mixed factor ANOVA assessed the number of trials needed to meet criterion performance in the SD and CD with within subjects factor of stage (SD v CD; 2 levels) and between subjects factors of lesion (DA v Sham; 2 levels) and sex (male v female; 2 levels). The CD is the first instance in which the subjects have to focus attention on a specific stimulus attribute while previously reinforced stimulus attributes are present.

An assessment of the ability to form an attentional set was assessed in a separate mixed factors ANOVA. Here, performance in the ID was compared to the ED with a within subjects' factor of stage (ID v ED; 2 levels) and between subjects factors of lesion (DA v Sham; 2 levels) and sex (male v female; 2 levels). Additionally, previous data has shown that if an attentional set was formed, there should be a decrease in trials to criterion from the CD to the ID. Here, animals should be able to maintain attention on the previously rewarded stimulus dimension from the CDR when the changeover occurs, leading to fewer trials to criterion in the ID. To assess this we used planned comparison, paired samples t-tests to compare ID vs. ED and CD vs. ID performance for subjects with Sham-LX and DA-LX.

In a third mixed factors ANOVA, the impact of ACC DA-LX on reversal learning was assessed where performance on all reversal stages were compared with a within subjects factor of stage (CDR, IDR, EDR; 3 levels) and between subjects factors of lesion (DA v Sham; 2 levels) and sex (male v female; 2 levels). Previous data has shown that all animals performance is worst on the first reversal (Newman & McGaughy, 2011).

As there were no significant main effects or interactions of sex, sex was pooled across lesion groups in order to assess the effects of changing the never relevant stimulus dimension. An independent samples t-test assessed the trials to criterion on the LI with a within subjects' factor of lesion (DA-Lx, Sham-Lx; 2 levels)

Results

In the analysis of conditional discriminations without (SD) and with distracting attributes (CD), we found that all subjects required more trials to criterion on the CD than the SD (stage $F(1, 29) = 29.30$, $p < 0.01$). The effects of the DA-LX increased susceptibility to distraction as these subjects required more trials to criterion on the CD, but not the SD (Stage x Lesion: $F(1, 29) = 6.06$, $p = 0.02$; Sham-LX vs. DA-LX; SD: $t(31) = -0.76$, $p = 0.45$; CD: $t(31) = -3.28$, $p < 0.01$). There was no significant main effect of sex or any interactions by lesion or stage (all $p > 0.01$). There was no main effect of lesion ($p > 0.05$).

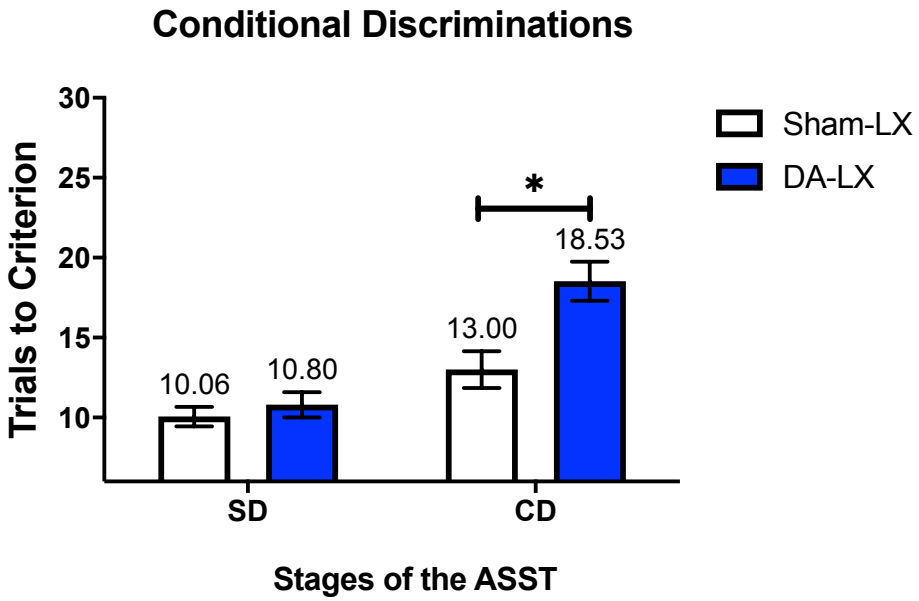

Figure 2.4: Subjects with DA-LX to the ACC (blue bars; n = 15) show impaired performance on the CD, but not SD stage of the ASST compared to animals with Sham-LX to the ACC (white bars; n = 18). Means are displayed above each bar. Error bars indicate the SEM.

In the analysis of a shift of attentional set in the ASST there was a main effect of stage ($F(1, 29) = 6.72$, $p = 0.02$), so all rats required fewer trials to reach criterion on the ID than the ED. There was also a main effect of lesion ($F(1, 29) = 28.44$, $p < 0.01$) revealed that DA-LX subjects required more trials to criterion on both the ID and the ED compared to subjects with Sham-LX (Sham-LX vs. DA-LX ID: $t(31) = -5.02$, $p < 0.01$; ED: $t(31) = -3.11$, $p < 0.01$; $\alpha = 0.03$). There were no other significant interactions by lesion (all $p > 0.05$). There were no significant main effects of sex or interactions with lesion or stage (all $p > 0.05$). Planned comparisons showed that subjects with Sham-LX, but not DA-LX had an increase in trials to criterion on the ED compared to the ID (ID vs. ED; Sham-LX: $t(17) = -2.97$, $p = 0.01$; DA-LX: $t(14) = -0.89$, $p = 0.39$;). And that subjects with Sham-LX, but not DA-LX, showed a decrease in trials to criterion at the ID compared to the CD (CD vs. ID; Sham-LX: $t(17) = 3.03$, $p = 0.01$; DA-LX: $t(14) = 1.72$, $p = 0.11$).

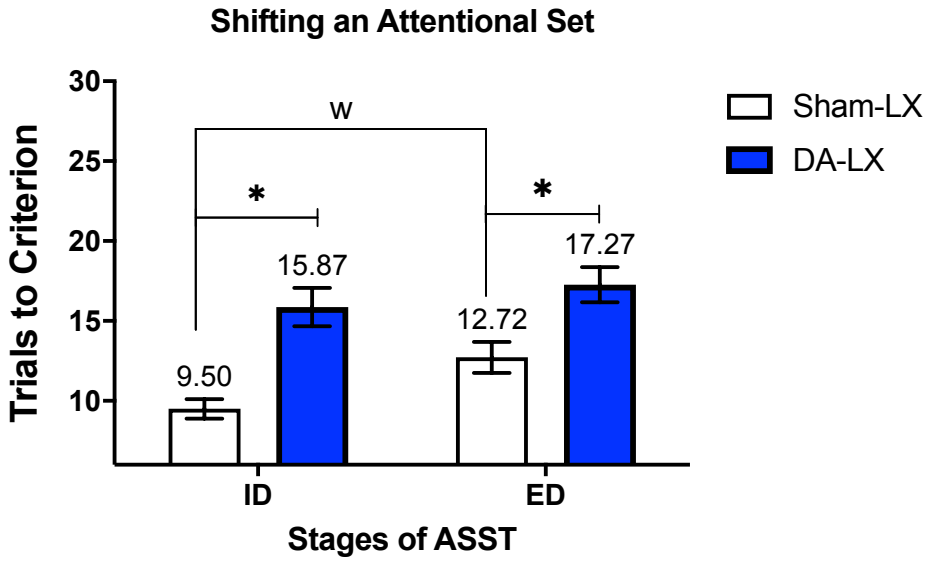

Figure 2.5: Subjects with DA-LX (blue bars, n =15) show an increase in trials to criterion at both the ID and ED stages of the ASST compared to subjects with Sham-LX (white bars, n =18). Sham-LX, but not DA-LX subjects show an increase in trials to criterion at the ED compared to the ID stage (as denoted by the w). Means are displayed above each bar, error bars are SEMs.

In the analysis of the reversal stages of the ASST there was a main effect of lesion ($F(1, 29) = 10.35$, $p < 0.01$), where subjects with DA-LX required more trials to criterion on all reversal stages (CDR, IDR and EDR) compared to subjects with Sham-LX. As has been seen in previous work(Newman & McGaughy, 2011), there was a main effect of stage ($F(2, 26) = 19.67$, $p < 0.01$) where all animals performance was worst on the first reversal (CDR) (CDR vs. IDR: $t(32) = 4.70$, $p < 0.01$; CDR vs EDR: $t(32) = 4.96$, $p < 0.01$; IDR vs. EDR: $t(32) = 1.25$, $p = 0.22$). There was no significant interaction of lesion by stage ($p > 0.05$). There was no significant main effect of sex or any interactions by lesion or stage (all $p > 0.05$).

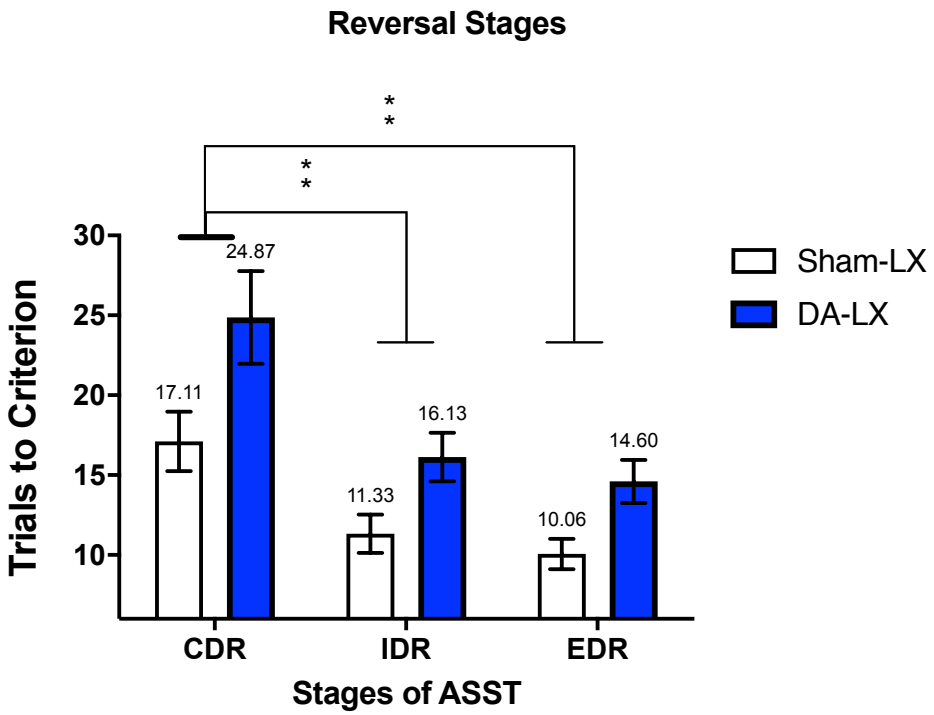

Figure 2.6: Subjects with DA-LX (blue bars, n = 15) show increased trials to criterion at all conditional reversal learning stages in the ASST compared to subjects with Sham-LX (white bars, n = 18). All subjects showed greater trials to criterion on the first reinforcement reversal compared to subsequent reversals. Means are displayed above each bar, SEM is indicated by the error bar.

In the test of learned irrelevance stage of the ASST, Subjects with Sham-LX and DA-LX showed no difference in performance when the never relevant stimulus dimension was altered (Sham-LX vs. DA-LX; LI: $t(31) = -1.24$, $p = 0.08$).

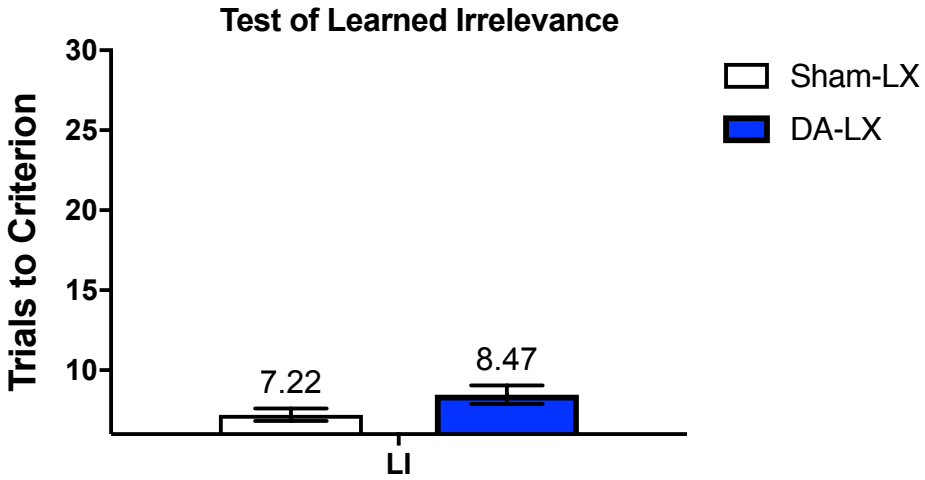

Figure 2.7: Sham-LX (white bar, n = 18) and DA-LX (blue bar, n =15) subjects showed no difference in trials to criterion when the never relevant stimulus dimension was altered. Means are displayed above each bar.

Discussion

Dopamine lesions of the ACC produced an increased susceptibility to distraction when stimulus attributes have been previously paired with reinforcement. In the ASST complex stimuli contain two stimulus dimensions which vary from the CD through to EDR. The CD is the first stage at which the stimuli become complex and contain multiple dimensions. The CD requires the continued attention of the stimulus dimension previously reinforced in the SD, but with the addition of irrelevant dimensions. The CD is the first stage that an irrelevant dimension is added. While all subjects required more trials to criterion when the irrelevant dimension was added, subjects with DA-LX required significantly more trials to criterion on the CD, indicating that

DA-LX subjects are less able to filter the newly introduced irrelevant information. All subjects showed proficient performance at each of the exemplars, and at the simple discrimination stage, showing proficiency for conditional discriminations when no distractors were present.

Deficits at the CD stage have been previously linked with ACC dysfunction (Newman & McGaughy, 2011) and has been seen in patients with schizophrenia (Jazbec et al., 2007; Pantelis et al., 1999). This particular deficit shows an inability to ignore previously reinforced attributes of a complex stimulus and thus increased susceptibility to distraction. During the exemplar stages, animals are taught that attention to any one of the three stimulus dimensions can elicit reward, as each of the three are tested in separate conditional discriminations. When the subjects reach the CD stage, the introduced stimulus dimension has a prior reinforcement history from the exemplar stages (Newman & McGaughy, 2011). This prior reinforcement history is what appears to be driving the increased susceptibility to distraction in the CD stage of the task.

The overarching increase in trials to criterion for DA-LX subjects compared to Sham-LX subjects across all stages of the task except the SD and LI stages may indicate that DA-LX subjects were unable to update reinforcement contingencies effectively once stimuli became complex in the ASST. Each stage of the task requires the ability to use reinforcement information to guide decision making. When reinforcement reversals occur, subjects had to use reinforcement cues to make a change in behavior. When the previously rewarded stimuli no longer predicted reward, subjects needed to adjust accordingly. As has been seen previously, all subjects require more trials to criterion on the first reinforcement reversal (CDR) compared to subsequent reversals (IDR, EDR) (Newman & McGaughy, 2008a, 2011). Here, DA-LX subjects showed this same pattern of behavior, however, they required significantly more trials to criterion compared to Sham-LX subjects to do so, showing an inability to integrate prior

outcome history with action judgments to make optimal decisions (Hyman et al., 2017; Hyman et al., 2012; Kennerley et al., 2006; Laurens et al., 2003).

Subjects with dopamine lesions of the ACC were not only distracted by previously reinforced attributes of a complex stimulus, but they also did not form an attentional set. When an attentional set is formed, you can see both the benefit of having formed an attentional set and the cost of having formed an attentional set. The benefit of a set is seen in a difference in performance on the ID compared to the CD. The same stimulus dimension is reinforced in the SD, CD, and ID stages. If a set has been formed, it should require fewer trials to meet criterion in the ID, compared to the CD, as the subject should continue to attend to the dimension rewarded in the CD, despite the total changeover design. Subjects with Sham-LX showed this decrease in trials to criterion on the ID compared to the CD, but subjects with DA-LX did not. The cost of having formed an attentional set can be seen in the difference in performance on the ID compared to the ED stages of the task. At the ED, the reinforced stimulus dimension changes, this is the stage at which subjects must shift their attentional set. As the previously reinforced stimulus dimension no longer predicts reward, subjects should require more trials to reach criterion on the ED compared to the ID, showing the cost of having formed an attentional set. Sham-LX subjects show this difference in the ID and ED stages, but DA-LX subjects do not. The lack of CD/ID and ID/ED difference in the DA-LX subjects suggests that these animals are unable to form an attentional set (McGaugh y et al., 2008; Newman & McGaughy, 2008a, 2011, 2011a).

CHAPTER III

THE IMPACT OF DOPAMINERGIC DEAFFERENTATION OF THE ANTERIOR CINGULATE CORTEX IN A TASK OF SUSTAINED ATTENTION

The ability to sustain attention is a part of daily life. From Mackworth's literature on the life or death necessity while monitoring radars (1948), to pass or fail necessity in academic settings, the ability to maintain attention over long periods is crucial to success. Deficits in sustained attention are often seen in children (Demeter et al., 2013) and patients with ADHD (Barkley, 1997); all of which are thought to be impacted by dopaminergic dysfunction in the ACC (Maciejewski, Brieant, Lee, King-Casas, & Kim-Spoon, 2020; Pantelis et al., 1997; Seidman et al., 2006). The ACC is crucial for sustained attention in several ways. One of which is in preparatory attention, maintaining focus during the intertrial interval or scanning phase of the task (Wu et al., 2017), as well as in maintaining signal accuracy across time (Newman & McGaughy, 2011). While the SAT and variant sessions provide valuable information about subjects' cognitive deficits in their own right, they also provide a method of unpacking the deficits seen in the ASST.

In the ASST DA-LX subjects showed increased susceptibility to distraction to stimuli with a previous reward history. In the sustained attention task (SAT) subject's ability to filter distracting stimuli with no prior reinforcement history can be assessed (McGaughy & Sarter, 1995; Newman & McGaughy, 2011). Further, since the ASST is self-paced, it is not well suited to assess cognitive fatigue which has previously been associated with dysfunction of the ACC (Newman & McGaughy, 2011). In experiment 2, all subjects were assessed in the sustained

attention task (McGaughy & Sarter, 1995), and five variant sessions, which assessed specific distractor conditions or alterations in the relationship between response and reinforcement (Demeter et al., 2013; McGaughy & Sarter, 1995; Newman & McGaughy, 2008b, 2011). All stages of the ASST contain a complex stimulus, and therefore a predicted element of distraction (Newman & McGaughy, 2011). As dopaminergic dysfunction in the ACC is hypothesized to increased susceptibility to distractors with a history of prior reinforcement, further exploration of each detriment is needed without distractors with a reinforcement history, which is examinable in the SAT.

Based on the deficits seen in the ASST, subjects were tested in five SAT variants. As DA-LX subjects showed an inability to ignore previously reinforced distractors, a novel distractor with no reinforcement history was used. In this variant, seen in previous literature, a 0.5Hz flashing houselight is present throughout the entire testing session (McGaughy & Sarter, 1995; Newman & McGaughy, 2008b, 2011). The flashing houselight is never paired with reinforcement. The ACC has been shown to be sensitive to cross-modal stimuli in human patients with schizophrenia (Laurens et al., 2005), however, ACC IBO-LX rodents did not show this same sensitivity to cross-modal distraction (Newman & McGaughy, 2011), but rodents with lesions to the PL do (Newman & McGaughy, 2008b). As dopaminergic dysfunction is thought to be related to the increased attention paid to novel stimuli in schizophrenia (Dolan et al., 1995), the present study may serve to close the gap in the contrasting findings. To assess the impact of DA-LX of the ACC on increased susceptibility to cross-modal distraction, subjects were also exposed to a session in which an auditory distractor was presented with the same timing as the unimodal distractor session (0.5Hz tone). If the novel distractors failed to discriminate the lesion

groups, it would indicate that DA-LX subjects are not more susceptible to all distraction, but rather elements of complex stimuli which have a prior reinforcement history.

In the ASST, DA-LX subjects also showed deficits at the reversal stages which could have been due to an inability to ignore the distracting dimensions of the complex stimulus, or due to an inability to update reinforcement contingencies. To discriminate which was impacted by DA-LX, subjects were tested in sessions where reinforcement was delayed predictably (2 seconds after a correct response), unpredictably (0, 1, or 2s following a hit or correct rejection), or removed (no reinforcement regardless of accuracy). If DA-LX subjects showed an ability to update reinforcement contingencies when no distractors were present it would point to the detriment at the reversal stages in the ASST being driven in some part by the distractibility element of the task. If the ability to update reinforcement contingencies was different between the lesion groups, it would indicate that DA-LX of the ACC produced a deficit in utilizing reinforcement feedback to make optimal decisions.

Research Design and Methods

General Procedure

Following testing in the ASST, subjects began training for the sustained attention task. All animals were returned to training to lever press for one day to confirm criterion performance had been maintained. All testing occurred 6-7 days/week from 11:00am – 3:00pm, with each session running at the same time each day. Animals remained food restricted and were tested in the same operant chamber for the entire duration of testing. Following completion of the removal of reward variant, subjects were eligible for euthanasia and histological processing.

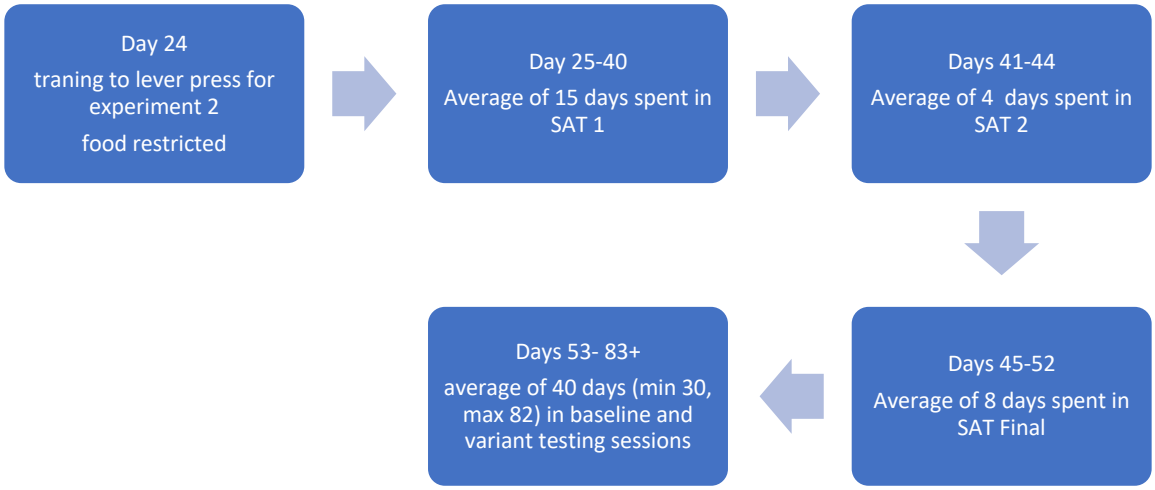

Figure 3.1: timeline of the procedure continued from experiment 1 in chapter 2. Experiment 2 began with training subjects for the sustained attention task and ended with completion of all task variants.

Behavior

Apparatus and Materials: See Chapter 2 methods

Training

Shaping to lever press: See description in Chapter 2 methods.

Signal and Non-Signal Response Rule Acquisition: In the next phase of training, rats are introduced to stimulus and non-stimulus presentations. Prior to the start of the session animals are acclimated to the operant chamber for 1min in darkness. Throughout the session the house light is illuminated and the intertrial interval is 12±3 seconds. In order to facilitate the association between the signal trials and the correct lever response in this stage, signal trials are presented

such that the panel light over the correct lever (left panel light) in addition to the target central panel light are illuminated for 1s. after a signal or non-signal presentation there is a 2sec delay, after which both levers extend into the chamber. Animals have 4s after the levers extend to make a selection or that trial is recorded as an error of omission, and the inter-trial-interval (ITI) is reinstated. Following any lever depression or 4s both levers are retracted. During a signal trial a left lever depression is a "hit" and is rewarded with food, a right lever depression is a "miss" and reinstates the ITI followed by a correction trial (McGaughy & Sarter, 1995; Parasuraman, Masalonis, & Hancock, 2000). A correction trial is a repetition of the previous trial type (signal or non-signal). When three incorrect correction trial responses or errors of omission occur, a forced trial was initiated. In a forced trial the signal or non-signal will co-occur with only the correct lever extended until the lever is selected, with a 90s limited hold. During a non-signal trial, a left lever selection is counted as a "false alarm" and the correction trial and forced trials as described above will occur (Parasuraman et al., 2000). A right lever selection is counted as a "correct rejection" and is rewarded with food before the ITI is reinstated (Parasuraman et al., 2000). Animals must meet >70% correct signal trials, >70% correct non-signal trials, and over 50 responses for two consecutive testing sessions to be eligible to progress to the next stage of training. Each session consists of 160 trials, or a maximum time of 45min, at which point the session will end and the houselight will turn off.

Final task acquisition with targets of central panel light: In the subsequent phase of training, the left panel light presentation during signal trials is eliminated. All other aspects of the task are the same as described above. Animals must meet >70% correct signal trials, >70% correct non-

signal trials, and over 50 responses for two consecutive testing sessions to be eligible to progress to the final training stage.

Sustained Attention Task: In the final version of the task animals must continue to discriminate between signal and non-signal stimulus presentations. Signal presentations are now embedded in a dynamic stimulus range, where signals are 500, 100, or 25ms in duration(Figure 3.1). Each session consisted of 162 trials, broken into three blocks. In each block there were 9 presentations of each stimulus duration, and 27 non-signal trials in each block.

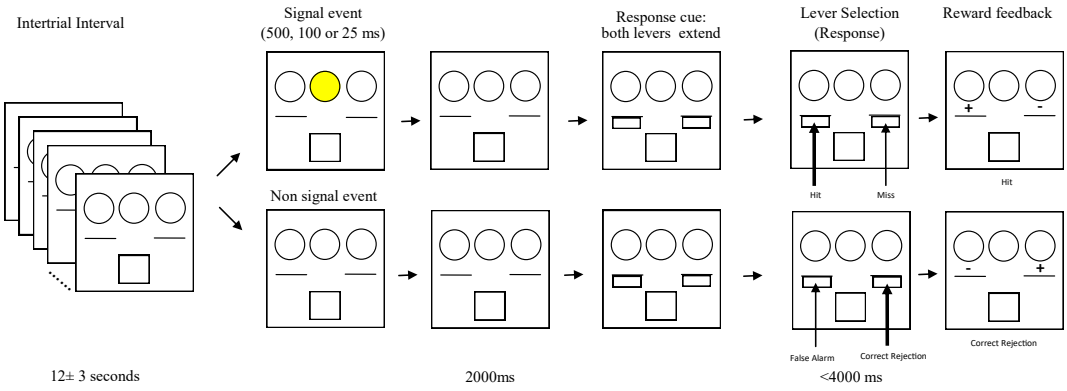

Figure 3.2: schematic of the sustained attention task, where the squares represent the front wall of the operant chamber which houses the light panel, levers, food port, and tone generator.

Cognitive Variants

After successful acquisition of the baseline task, animals began running variant sessions. Preceding each variant session animals were required to meet two consecutive days of criterion performance in the baseline sustained attention task. After this criterion was met, subjects were

run in a variant session. After the variant session subjects were returned to the SAT until criterion was met again. To prevent testing effects, the order in which animals were run in the 0.5Hz light, 0.5Hz tone, predictable delay, and unpredictable delay were counterbalanced and matched between groups. All animals performed removal of reinforcement last to prevent any increase in omissions to carry over into subsequent testing sessions. Following completion of all variant sessions, animals were euthanized, and brain tissue was prepared for histological processing.

Unimodal Distractors: Previous work has shown that all animals ability to detect signals is hindered when the flashing houselight is present (McGaughy & Sarter, 1995). Animals were tested with a predictable light (0.5Hz flashing), throughout the testing session.

Cross modal distractors: The ACC has been shown to activate when cross modal distractors are present (Laurens et al., 2005). As such animals were tested in variant sessions where tone was presented in the same manner at the unimodal distractor session, predictable (0.5Hz) throughout the testing session.

Reinforcement Variants: The ACC has been linked to updating reinforcement contingencies (Kennerley et al., 2006), while dopamine has also been shown to have a bidirectional response where better reward shows an increase in firing, predicted reward shows no firing, and a lack of reward when a reward was expected shows a depression in firing rate (Chang et al., 2016; Schultz, 1997; Steinberg et al., 2013). The exact time at which reinforcement appears following an action has been shown to influence dopamine firing as well, where firing decreases

specifically when the expectation of reinforcement is violated (when no reward is received), but will increase at a delay point if reinforcement is delayed (Hollerman & Schultz, 1998). If this reward is delayed in a predictable manner, firing patterns will alter as the new reward timing is learned, and if reward is removed, firing will also normalize as the action-outcome association changes. In the current study, reward was delayed predictably (2s following a selection), unpredictably (0, 1, or 2s following a selection), or removed (no reward given regardless of accuracy). If reinforcement contingencies were updated, subjects would be expected to show the same accuracy for signal trials as they did in the baseline session, indicating that the error signal seen in the ACC is not required for updating reinforcement contingencies when no distractors are present.

Statistical Analysis

From each session the number of hits, misses, correct rejections, false alarms, and errors of omission were recorded, totaling 162 trials per session. These 162 trials were separated into three blocks where block 1 contained trials 1-54, block 2 contained trials 55- 109, and block 3 contained trials 110-162 (54 trials each) in order to assess the impact of time on task. Each block contained an equal number of signal, and non-signal trials (9 of each signal length and 27 non-signal trials). The hit accuracy (hits/(hits + misses)) was calculated for each signal length (500, 100, 25ms) (McGaughy & Sarter, 1995). The correct rejection accuracy (correct rejections/(correct rejections + false alarms)) was also calculated (McGaughy & Sarter, 1995). All statistical analyses were conducted using SPSS 26.0 (SPSS, Chicago, IL) for both MacOS and Windows. In the case of a violation of sphericity, the degrees of freedom were corrected

using the Huynh-Feldt correction. Epsilon (ε) values not equal to 1 are reported below. A Bonferroni correction was used to modify alpha levels for multiple comparisons. For the analysis of the 0.5Hz tone, one further dopamine lesioned female subject was removed from the analysis for having not met criterion in the baseline session preceding the 0.5Hz tone session.

SAT sessions

All SAT sessions preceding variant sessions (5) were analyzed in two repeated measures analysis of variance (ANOVAs). The effects of signal length (3), time on task (3 blocks of 54 trials/each), and day (5) on hit accuracy were analyzed in a mixed factors ANOVA with two between subjects factors (Lesion, 2 levels; Sex, 2 levels). A separate mixed factor ANOVA was conducted to assess non-signal trial accuracy with two between subjects' factors (Lesion, 2 levels; Sex, 2 levels), and two within subjects' factors (Day, 5 levels; Block, 3 levels).

Variant sessions

Due to sex differences in the baseline SAT reported in detail below, each analyses of variant sessions was separated by sex. The SAT session prior to each variant session served as the baseline so all task variant analyses include the factor of day (2). Separate mixed factors ANOVA's were conducted for each sex for each variant. Where for hits accuracy, there was one between subjects' factor (Lesion, 2 levels) and three within subjects factors (Day, 2 levels; Block, 3 levels; Signal, 3 levels). And for correct rejection accuracy there was one between subjects factor (Lesion, 2 levels) and two within subjects factors (Day, 2 levels; Block, 3 levels).

Results

Baseline Accuracy on Signal Trials

There was little difference in baseline performance between sham-LX and DA-LX subjects. Baseline accuracy for signal trials increased for all subjects over repeated testing sessions ($F(4, 116) = 4.06$ $p < 0.01$). As has been shown in previous work, all subjects showed signal dependent performance, where accuracy was highest for longer signal durations ($F(2, 58) = 423.90$, $p < 0.01$). The effects of signal length interacted with time on task ($F(4, 116) = 4.56$, $p < 0.01$) where accuracy for 500msec signals declined in block 3, 100msec signals declined in block 2, and 25msec signals was stable across the session. The effects of block and signal interacted with lesion and sex ($F(4, 116) = 2.66$, $p = 0.03$). Further analysis (corrected $\alpha < 0.01$; 12 comparisons) revealed that DA-LX females, but no other group, did not show signal dependent performance in block 3 (500msec vs. 100msec: $t(6) = 2.52$, $p = 0.05$; 100msec vs. 25msec: $t(6) = 3.45$, $p = 0.01$). Sham-LX males, DA-LX males, and Sham-LX females showed signal dependent performance in all blocks (all $p < 0.0003$). For means see appendix A.

When the presence of a baseline side bias was assessed (0.5 = equal right and left lever selections, 0.0 = 100% non-signal lever selections, 1.0 = 100% signal lever selections), a session by block by lesion interaction was revealed for male subjects ($F(8, 128) = 3.12$, $p < 0.01$). Sham-LX males showed an increased non-signal side bias compared to DA-LX males in session 4 in block 3 (Sham-LX vs. DA-LX: baseline 4, block 3: $t(16) = 2.61$, $p = 0.02$; Sham-LX: 0.39±0.03; DA-LX: 0.51±0.04). DA-LX males showed an increased non-signal side bias compared to Sham-LX males in session 5 in the first block (Sham-LX vs. DA-LX ; baseline 5, block 1: $t(16) = 2.54$, $p = 0.02$; Sham-LX: 0.47±0.03; DA-LX: 0.36±0.03). However, these did not remain

significant with the corrected $a < 0.01$ for 5 comparisons. Females showed an interaction of session and lesion ($F(4, 52) = 5.82$, $p < 0.01$). Further analysis showed a significant difference on baseline day 1 (Sham-LX vs. DA-LX: $t(12) = 3.01$, $p = 0.01$; all other $p > 0.05$; sham-LX: 0.47±0.02; DA-LX: 0.39±0.01), however, these also did not hold to the corrected $a < 0.01$ for 5 comparisons.

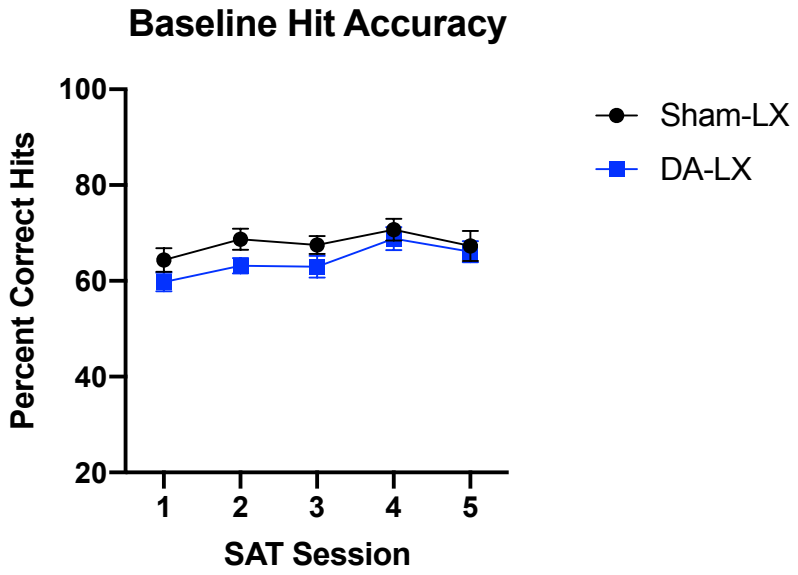

Figure 3.3: All subjects show increased accuracy on signal trials with repeated SAT testing sessions.

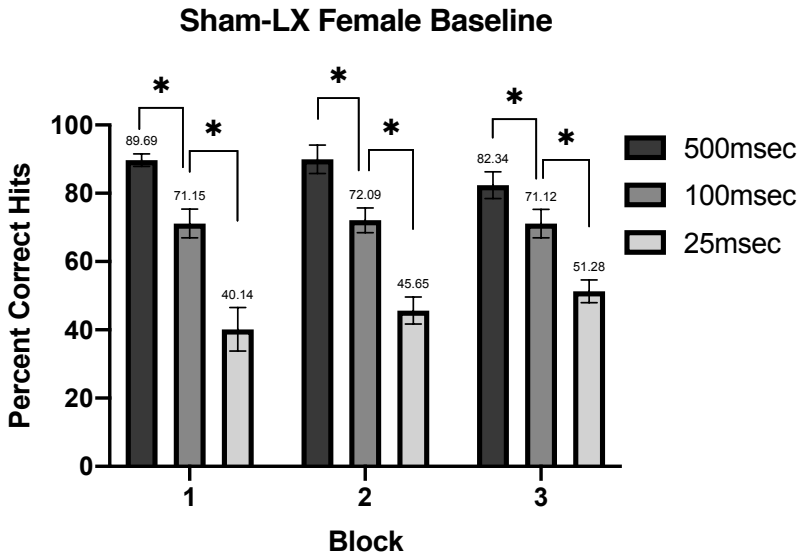

Figure 3.4: Sham-LX females showed signal dependent performance where accuracy was higher for 500msec signal presentations (dark gray bars) compared to 100msec presentations (medium gray bars), and accuracy for 100msec presentations was higher than 25msec presentations (light gray bars) across time on task. * indicates $p < 0.003$, means are displayed above each bar, error bars are SEMs

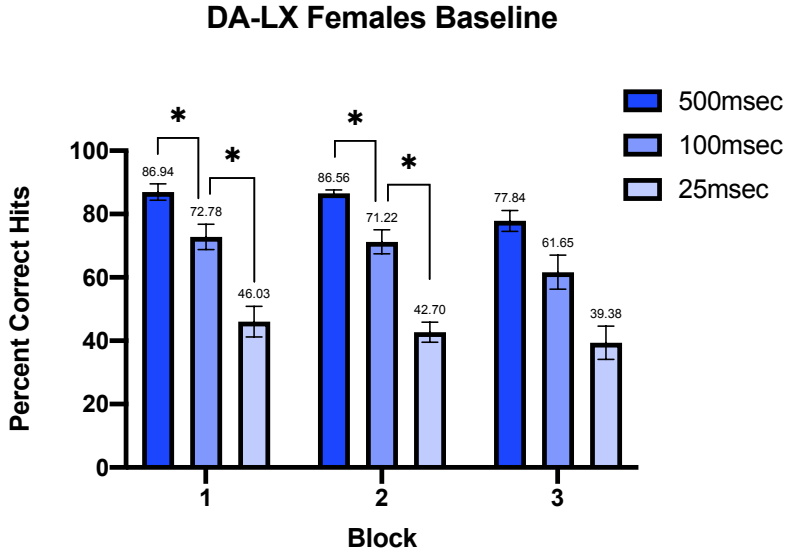

Figure 3.5: DA-LX females showed signal dependent performance where accuracy was higher for 500msec signal presentations (dark blue bars) compared to 100msec presentations (medium blue bars), and accuracy for 100msec presentations was higher than 25msec presentations (light blue bars) in blocks 1 and 2, but not 3. * indicates $p < 0.003$, means are displayed above each bar, error bars are SEMs

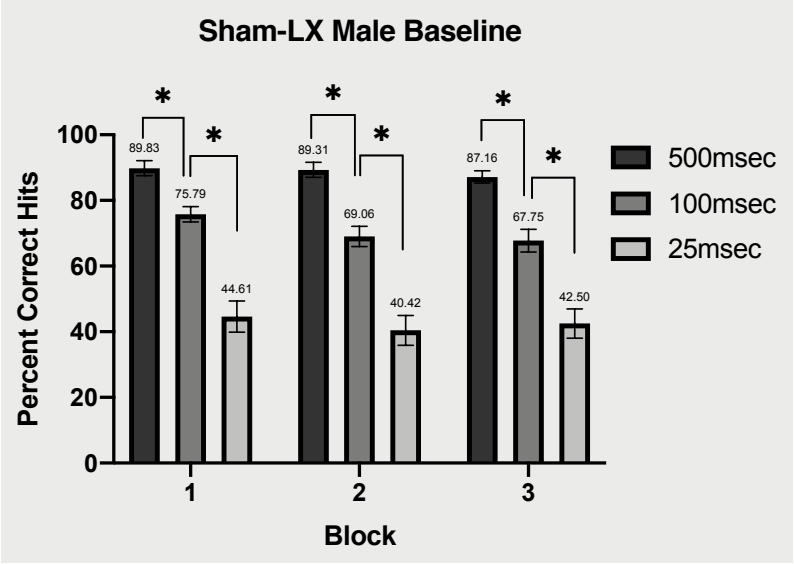

Figure 3.6: Sham-LX males showed signal dependent performance where accuracy was higher for 500msec signal presentations (dark gray bars) compared to 100msec presentations (medium gray bars), and accuracy for 100msec presentations was higher than 25msec presentations (light gray bars) across time on task. * indicates $p < 0.003$, means are displayed above each bar, error bars are SEMs.

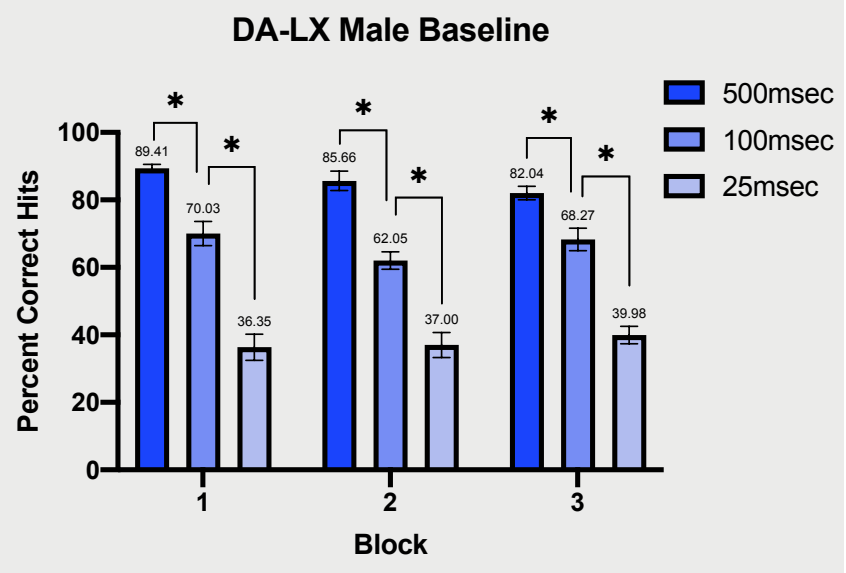

Figure 3.7: DA-LX males showed signal dependent performance where accuracy was higher for 500msec signal presentations (dark blue bars) compared to 100msec presentations (medium blue bars), and accuracy for 100msec presentations was higher than 25msec presentations (light blue bars) across time on task. * indicates $p < 0.003$, means are displayed above each bar, error bars are SEMs.

Baseline Accuracy on Non-Signal Trials

There was little difference in baseline performance between sham-LX and DA-LX subjects for non-signal trials. All subjects showed improvement on non-signal trial accuracy with repeated sessions ($F(4, 116) = 6.62$, $p < 0.01$). There was a main effect of time on task ($F(2, 58) = 14.64$, $p < 0.01$), where accuracy for non-signal trials declined with prolonged testing. The effect of time on task interacted with lesion and sex ($F(2, 58) = 4.89$, $p = 0.02$). Further analysis (corrected $\alpha = 0.01$; 8 comparisons) showed that male subjects with DA-LX showed a decrease in accuracy for non-signal trials over time on task (block 1 vs. block 2: $t(7) = -0.62$, $p = 0.56$; block 2 vs. block 3: $t(7) = 3.46$, $p < 0.01$), but sham-LX did not (block 1 vs. block 2: $t(9) = -1.76$, $p = 0.11$; block 2 vs. block 3: $t(9) = 2.30$, $p = 0.05$). Females did not show any decline in accuracy for non-signal trials over time on task (Sham-LX: block 1 vs. block 2: $t(7) = -0.88$, $p = 0.41$; block 2 vs. block 3: $t(7) = 3.12$, $p = 0.02$; DA-LX: block 1 vs. block 2: $t(6) = 0.54$, $p = 0.61$; block 2 vs. block 3: $t(6) = 0.15$, $p = 0.89$). For means see appendix A.

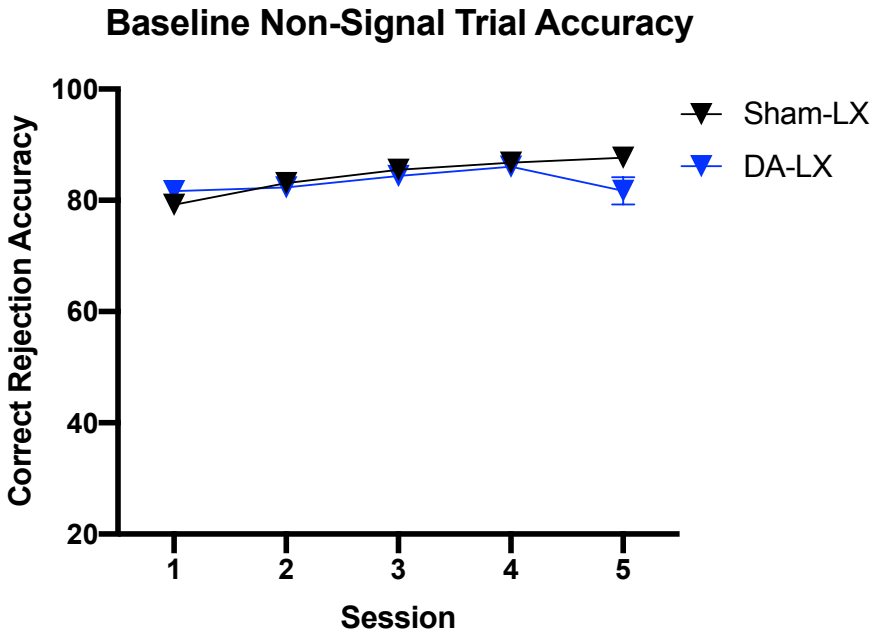

Figure 3.8: All subjects showed an increase in accuracy on non-signal trials with repeated SAT testing sessions.

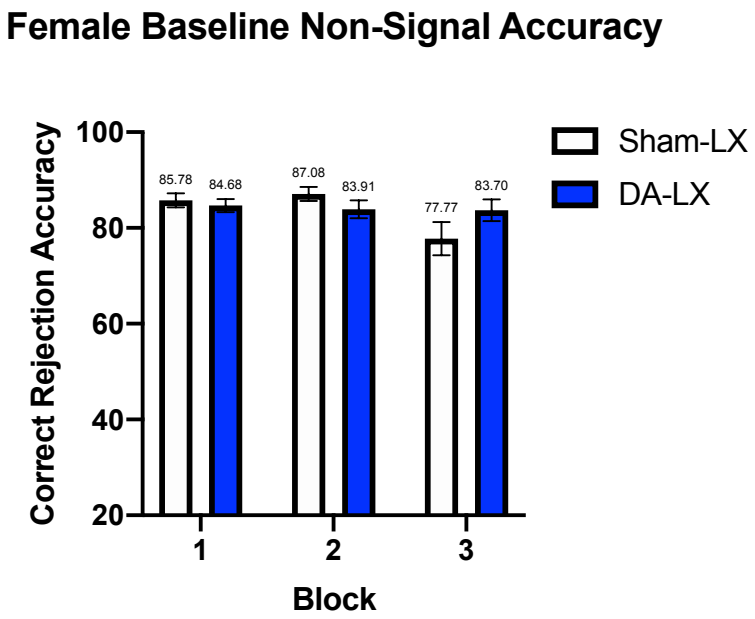

Figure 3.9: Both Sham-LX females (white bars, n = 8), and DA-LX females (blue bars, n = 7) show no decline in accuracy for non-signal trials over time on task. Means are displayed above each bar, error bars are SEMs.

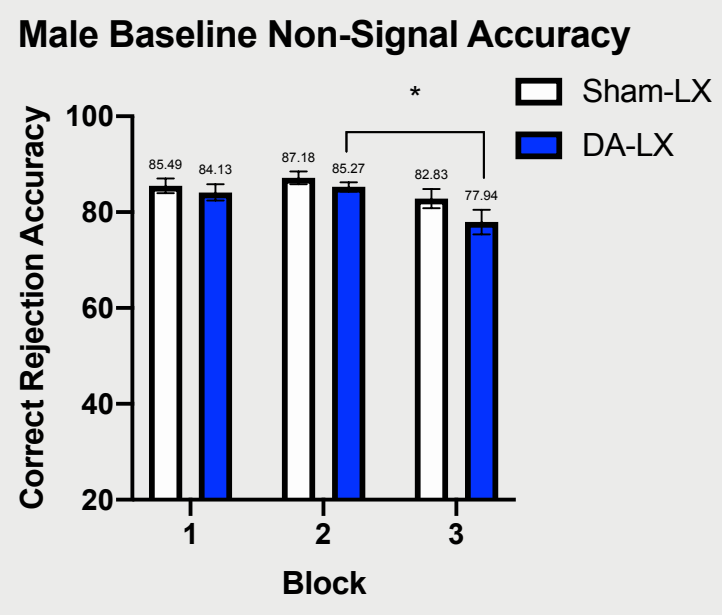

Figure 3.10: Sham-LX males (white bars, n = 10) , but not DA-LX males (blue bars, n = 8), showed a decline in accuracy for non-signals trials over time on task (block 3). means are displayed above each bar, error bars are SEMs, * indicates $p < 0.01$.

Unimodal Variant

0.5Hz Light Female Hits Accuracy: When the 0.5Hz flashing houselight was present all female subjects' accuracy for signal trials declined ($F(1, 13) = 101.25$, $p < 0.01$). The effects of the 0.5Hz flashing houselight interacted with signal length ($F(2, 26) = 11.29$, $p < 0.01$, $\varepsilon = 0.96$), where subjects accuracy for 500msec signal lengths declined (baseline vs. 0.5Hz light: $t(14) = 8.50$, $p < 0.01$), 100msec signal lengths declined (baseline vs. 0.5Hz light: $t(14) = 9.95$, $p < 0.01$) and 25msec presentations declined (baseline vs. 0.5Hz light: $t(14) = 2.89$, $p = 0.01$) (corrected $\alpha = 0.02$; 3 comparisons). The effects of the 0.5Hz flashing light and signal length interacted with dopamine lesion ($F(2, 26) = 3.75$, $p = 0.04$). Females with Sham-LX, but not DA-LX showed a decline in accuracy for all signals lengths compared to baseline signal trial accuracy (Sham-LX: baseline vs. 0.5Hz light: 500msec: $t(7) = 8.57$, $p < 0.01$; 100msec: $t(7) = 5.87$, $p < 0.01$; 25msec $t(7) = 3.20$, $p = 0.02$). DA-LX females only showed a decline in 500msec and 100msec signal presentations compared to baseline signal trial accuracy in the presence of the flashing houselight (DA-LX: baseline vs. 0.5Hz light: 500msec: $t(6) = 4.78$, $p < 0.01$; 100msec: $t(6) = 9.59$, $p < 0.01$; 25msec: $t(6) = 0.94$, $p = 0.39$). However, the Sham-LX change in 25msec signal accuracy was no longer significant with the correction for six comparisons (corrected $\alpha < 0.01$).

The presence of the 0.5Hz flashing houselight did not significantly increase errors of omission ($F(1, 31) = 2.18$, $p = 0.15$; baseline: $M = 0.66 \pm 0.13$; 0.5Hz light: $M = 0.88 \pm 0.22$). All females showed a side bias for the non-signal lever in the presence of the 0.5Hz light that was not present at baseline ($F(1, 13) = 22.97$, $p < 0.01$), this interacted with time on task ($F(2, 26) = 6.19$, $p < 0.01$) where all females bias for the left lever was present in blocks 2 and 3 (baseline vs. 0.5Hz light: b2: $t(14) = 6.39$, $p < 0.01$; b3: $t(14) = 4.27$, $p < 0.01$; B2: baseline: $M = 0.42 \pm 0.02$; 0.5Hz tone: $M = 0.27 \pm 0.03$; B3: baseline: B3: $M = 0.45 \pm 0.03$; 0.5Hz tone: M =

0.31±0.03), but not in block 1 (baseline vs. 0.5Hz light: b1: t(14) = 0.75, p = 0.47; baseline B1: M = 0.40±0.03; 0.5Hz light: M = 0.38±0.03; corrected α = 0.02; 3 comparisons). Side bias did not differ between the lesion groups (all p > 0.05).

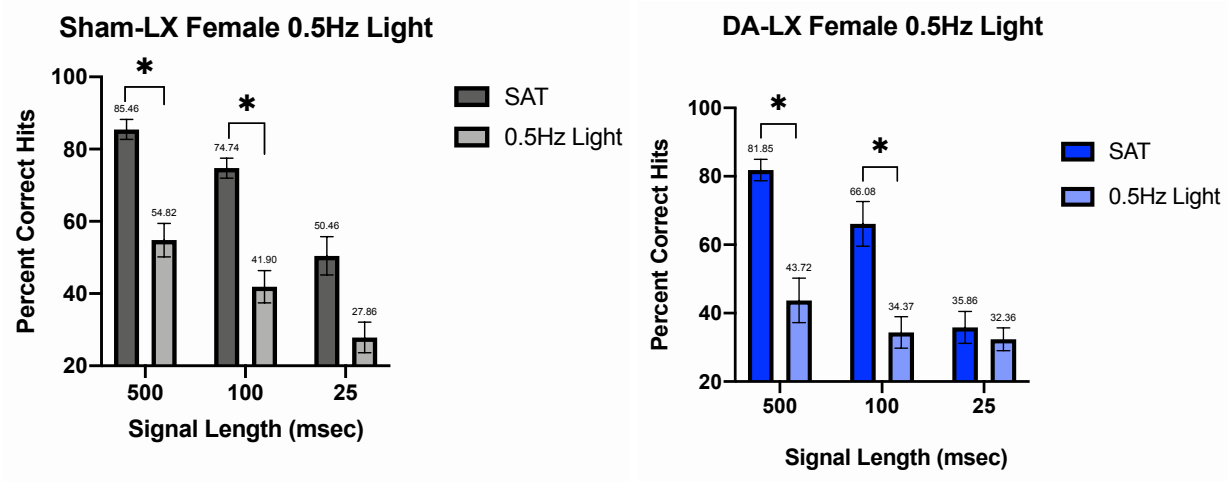

Figure 3.11: Sham-LX females showed a decline in accuracy for 500msec and 100msec signal targets when the 0.5Hz flashing houselight was present (light gray bars, n = 8) compared to baseline performance (dark gray bars, n = 8). DA-LX females showed a decline in accuracy for 500msec and 100msec signal targets when the 0.5Hz flashing houselight was present (light blue bars, n = 7) compared to baseline performance (dark blue bars, n = 7). means are displayed above each bar, error bars are SEMs, * indicates p < 0.01.

0.5Hz Light Female Correct Rejection Accuracy: As with signal trials, the presence of the 0.5Hz flashing light produced a decline in non-signal trial accuracy in all females (F(1, 13) = 7.60, p = 0.02). Unlike at baseline, the main effect of time on task (F(2, 26) = 3.57, p = 0.04) revealed an increase in all female subjects accuracy for non-signal trials in block 2 (block 1 vs. block 2: t(14) = -2.87, p = 0.01), and maintained accuracy for non-signal trials in block 3 (block 2 vs. block 3: t(14) = 2.08, p = 0.06) (corrected α = 0.03; 2 comparisons). The effects of the

0.5Hz flashing light interacted with time on task ($F(2, 26) = 13.56$, $p < 0.01$). Compared to baseline, all female subjects accuracy for non-signal trials declined at the start of the task when the 0.5Hz flashing houselight was present (baseline vs. 0.5Hz light; block 1: $t(14) = 4.89$, $p < 0.01$). However, over time on the task, all subject's accuracy for non-signal trials matched baseline performance (baseline vs. 0.5Hz light: block 2: $t(14) = 2.01$, $p = 0.06$; block 3: $t(14) = -0.69$, $p = 0.50$) (corrected $\alpha = 0.02$; 3 comparisons). Unlike signal-trial performance, the presence of the 0.5Hz light did not differentiate performance on the basis of lesion. Additionally, the presence of the 0.5Hz flashing light did not increase errors of omission for non-signal trials ($F(1, 13) = 0.49$, $p = 0.49$; baseline: $M = 2.79 \pm 0.44$; 0.5Hz light: $M = 3.19 \pm 0.58$).

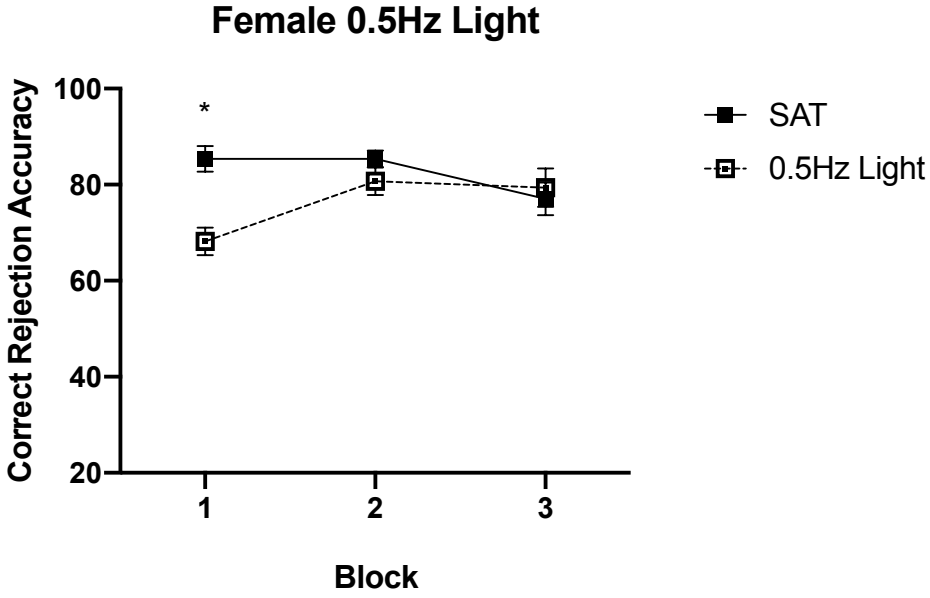

Figure 3.12: All females non-signal trials accuracy initially decreased (block 1) when the 0.5Hz light was present (closed black box and slid line; n =15; B1: M = 68.21 ± 2.88; B2: M = 80.73 ± 2.88; B3: M = 79.40 ± 4.02) compared to baseline performance (open box and dashed line, n = 15; B1: M = 85.36 ± 2.67; B2: M = 85.40 ± 1.73; B3: M = 77.05 ± 3.38). Accuracy for non-signal trials did not differ from baseline when the 0.5Hz flashing houselight was present in blocks 2 and 3. Error bars are SEMs, * indicates $p < 0.02$.

0.5Hz Light Males Hit Accuracy: Similar to females, all males showed a decline in accuracy for signal trials when the 0.5Hz flashing houselight was present ($F(1, 16) = 58.96$, $p < 0.01$). There was a main effect of time on task ($F(2, 32) = 10.91$, $p < 0.01$), which showed that unlike baseline, all males hit accuracy declined in block 2 (block 1 vs. block 2: $t(17) = 4.61$, $p < 0.02$), but did not decline further over time on task (block 2 vs. block 3: $t(17) = 0.12$, $p = 0.91$) (corrected $\alpha = 0.03$; 2 comparisons). The effects of the 0.5Hz light interacted with signal length ($F(2, 32) = 14.86$, $p < 0.01$) where all males showed a decline in accuracy for 500msec signal trials when the 0.5Hz light was present compared to baseline (baseline vs. 0.5Hz light: 500msec: $t(17) = 6.06$, $p < 0.01$) and 100msec signal trials (baseline vs. 0.5Hz light: $t(17) = 7.63$, $p < 0.01$), but not 25msec signals (baseline vs. 0.5Hz light: $t(17) = 1.10$, $p = 0.29$) (corrected $\alpha = 0.02$; 3 comparisons). Unlike in the female subjects, the 0.5Hz flashing houselight failed to discriminate performance between lesion groups on signal trials (all $p > 0.05$).

All males showed a slight side bias for the non-signal lever in the presence of the 0.5Hz light ($F(1, 16) = 6.09$, $p = 0.03$). There was a main effect of time on task ($F(2, 32) = 11.76$, $p < 0.01$) where the lever side bias was highest in block 2, however, the main effect of block did not interact with day ($F(2, 32) = 2.13$, $p = 0.14$; baseline: $M = 0.42 \pm 0.02$; 0.5Hz light: $M = 0.37 \pm 0.03$). The presence of the 0.5Hz flashing houselight did not increase errors of omission ($F(1, 16) = 1.21$, $p = 0.29$: baseline: $M = 0.16 \pm 0.08$: 0.5Hz light: $M = 0.32 \pm .20$).

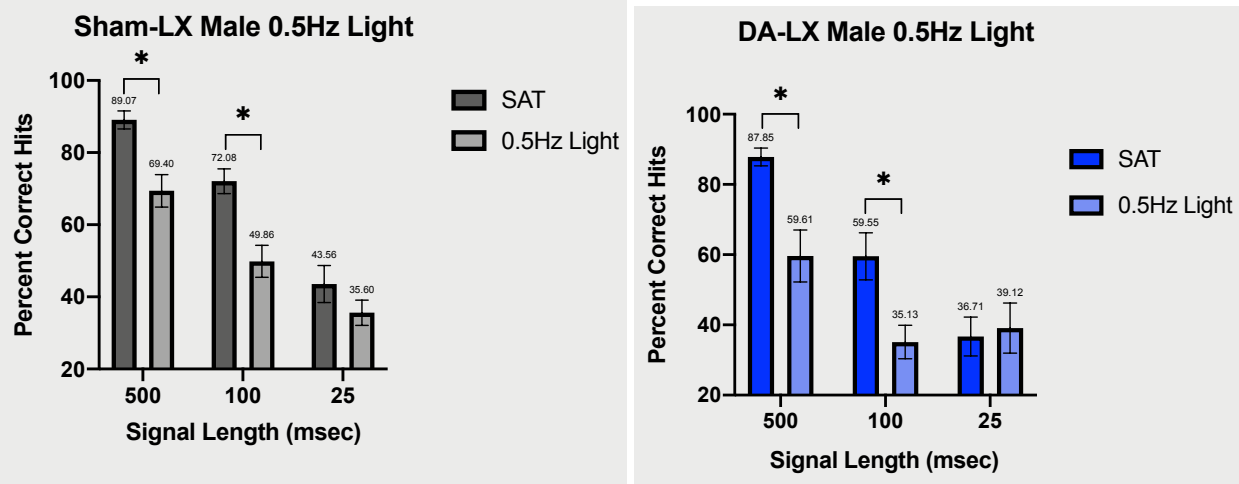

Figure 3.13: Sham-LX males (left, n = 10) and DA-LX males (right, n = 8) showed a decline hits accuracy for 500msec and 100msec signal presentations (combined across blocks) when the 0.5Hz flashing light was present (sham-LX; dark gray bars; DA-LX; dark blue bars) compared to baseline performance (sham-LX; light gray bars; DA-LX; light blue bars). However, there was no difference in accuracy for 25msec presentations between baseline and when the 0.5Hz flashing light was present. Means are displayed above each bar, error bars are SEMs.

0.5Hz Light Male Correct Rejection Accuracy: Males accuracy for non-signal trials declined when the 0.5Hz flashing houselight was present ($F(1, 16) = 11.55$, $p < 0.01$). Like the females, for males the main effect of time on task ($F(2, 32) = 7.23$, $p < 0.01$) showed an increase in accuracy for non-signal trials in block 2 (block 1 vs. block 2: $t(17) = -4.40$, $p < 0.01$), but no further decline in block 3 (block 2 vs. block 3: $t(17) = 2.18$, $p = 0.04$) (corrected $\alpha = 0.03$; 3 comparisons). The interaction of the effects of the 0.5Hz light and time on task ($F(2, 32) = 5.12$, $p = 0.01$), showed that like females, males accuracy for non-signal trials compared to baseline, was worse in block 1 (baseline vs. 0.5Hz light; block 1: $t(17) = 6.21$, $p < 0.01$), but was not difference from bassline performance over time on the task baseline vs. 0.5Hz light; (block 2: $t(17) = 1.50$, $p = 0.15$; block 3: $t(17) = 0.72$, $p = 0.48$) (corrected $\alpha = 0.03$; 3 comparisons). Unlike baseline, the presence of the 0.5Hz flashing light failed to discriminate performance

between the lesion groups (all $p > 0.05$). The 0.5Hz flashing light did not increase errors of omission ($F(1, 16) = 1.01$, $p = 0.33$; baseline: $M = 0.72 \pm 0.27$; 0.5Hz light: $M = 1.20 \pm 0.63$).

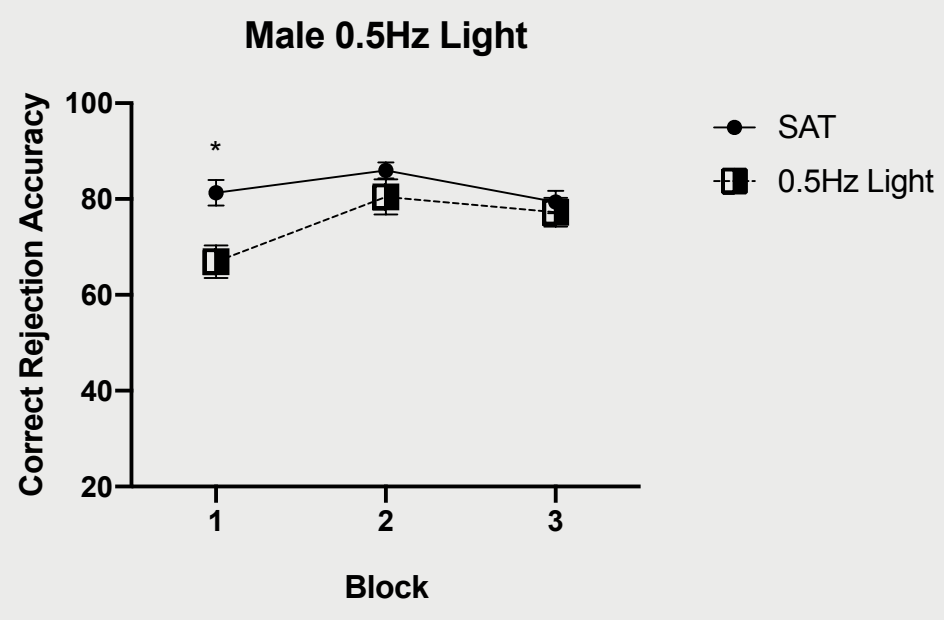

Figure 3.14: Males accuracy for non-signal trials initially decreased (block 1) when the 0.5Hz light was present (half open square and dashed line; n =18; B1: M = 66.90 ± 3.42; B2: M = 80.46 ± 3.68; B3: M = 77.25 ± 2.98) all compared to baseline (closed circle and solid line, n =18; B1: M = 81.30 ± 2.70; B2: M = 86.00 ± 1.70; B3: M = 69.40 ± 2.33). Males accuracy for non-signal trials did not differ from baseline accuracy over time on task (blocks 2 and 3). Error bars are SEMs, * indicates $p < 0.03$.

Cross Modal Variant

0.5Hz Tone Female Hits Accuracy: The presence of the 0.5Hz tone produced a decline in signal trial accuracy ($F(1, 12) = 14.17$, $p < 0.01$). This interacted with dopamine lesions ($F(1, 12) = 5.90$, $p = 0.03$). Further analysis showed that DA-LX females accuracy for signal trials was significantly lower than Sham-LX subjects at baseline (collapsed over time on task and signal length) (Sham-Lx vs. DA-LX: $t(12) = 2.85$, $p = 0.02$), but there was no difference between the

lesion groups in the presence of the 0.5Hz tone (Sham-LX vs. DA-LX: t(12) = 0.30, p = 0.77) (corrected α = 0.03; 2 comparisons). The decline in accuracy for signal trials was not due to an increase in errors of omission (F(1, 12) = 0.20, p = 0.66; baseline: M = 0.95 ± 0.18; 0.5Hz tone: M = 1.32 ± 0.29). All females showed a side bias for the non-signal lever in the presence of the 0.5Hz tone (F(1, 13) = 10.92, p < 0.01; baseline: M = 0.43 ± 0.01; 0.5Hz tone: M = 0.37 ± 0.02), and DA-LX females showed a consistently increased bias towards the non-signal lever compared to Sham-LX subjects (F(1, 13) = 6.07, p = 0.03; baseline; sham-LX: M = 0.47 ± 0.02; DA-LX: M = 0.39 ± 0.02; 0.5Hz tone; sham-LX: M = 0.39 ± 0.03; DA-LX: M = 0.35 ± 0.03). DA-LX females show a side bias that Sham-LX subjects do not at baseline in block 3 (Sham-LX vs. DA-LX; baseline; b1: t(12) = 0.31, p = 0.76; b2: t(12) = 1.35, p = 0.20; b3: t(12) = 3.07, p = 0.01; 0.5Hz tone; b1: t(12) = 0.57, p = 0.58; b2: t(12) = 0.59, p = 0.57; b3: t(12) = 2.14, p = 0.05) however, this failed to meet the corrected a < 0.01; 6 comparisons.

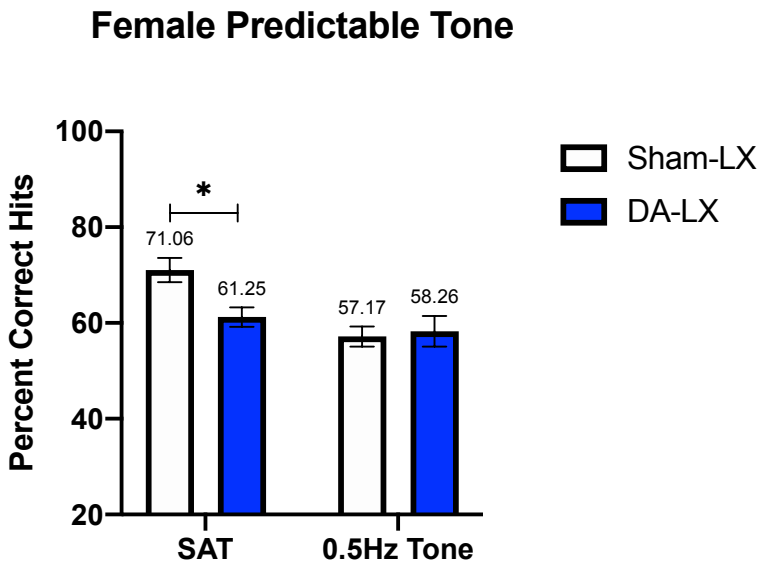

Figure 3.15: Females with DA-LX (blue bars, n = 6) show a decreased accuracy for signal trials (collapsed across signal lengths and blocks) compared to Sham-LX females (white bars, n = 8) at baseline, but not when the 0.5Hz tone was present. Means are displayed above each bar, error bars are SEMs, * indicated p < 0.03.

Predictable Tone Correct Rejection Accuracy Females: Unlike, the 0.5Hz light, the 0.5Hz tone failed to produce any deficits in non-signal accuracy ($F(1, 12) = 1.14$, $p = 0.31$), and no differences in performance based on lesion ($F(1, 12) = 2.62$, $p = 0.13$; all interactions of lesion with day and block $p > 0.05$).

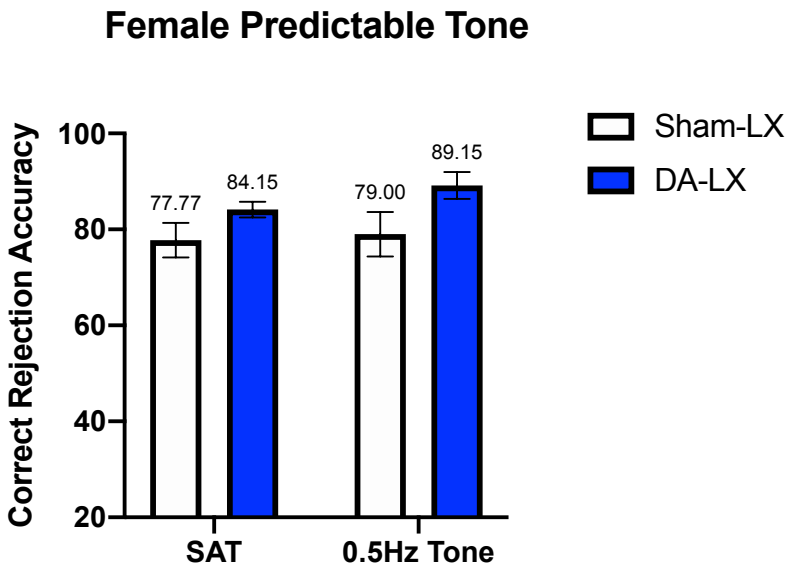

Figure 3.16: Neither Sham-LX females (white bars, n = 8) or DA-LX females (blue bars, n = 6) showed no change in accuracy on non-signal trials (collapsed across block) when the 0.5Hz tone was present compared to baseline performance. Means are displayed above each bar, error bars are SEMs.

Predicable Tone Hits Accuracy Males: Unlike the females, there was no main effect of day ($F(1, 16) = 0.94$, $p = 0.35$). The presence of the predictable tone did not facilitate a side bias in male subjects ($F(1, 16) = 0.09$, $p = 0.77$; baseline M = 0.42±0.02 0.5Hz tone M = 0.42±0.02).

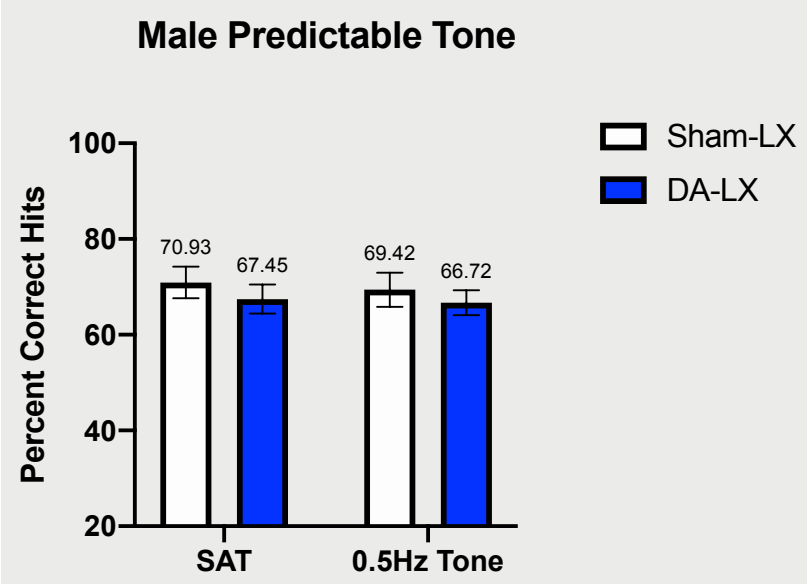

Figure 3.17: Neither Sham-LX males (white bars, n = 10) nor DA-LX males (blue bars, n = 8) showed no change in accuracy on signal trials (collapsed across signal lengths and block) when the 0.5Hz tone was present compared to baseline performance. Means are displayed above each bar, error bars are SEMs.

Predictable Tone Correct Rejection Accuracy Males: Like females, the 0.5Hz failed to impair accuracy for non-signal trials (day: $F(1, 16) = 1.56$, $p = 0.23$). Additionally, the 0.5Hz tone failed to differentiate non-signal trial accuracy between the lesion groups (all $p > 0.05$).

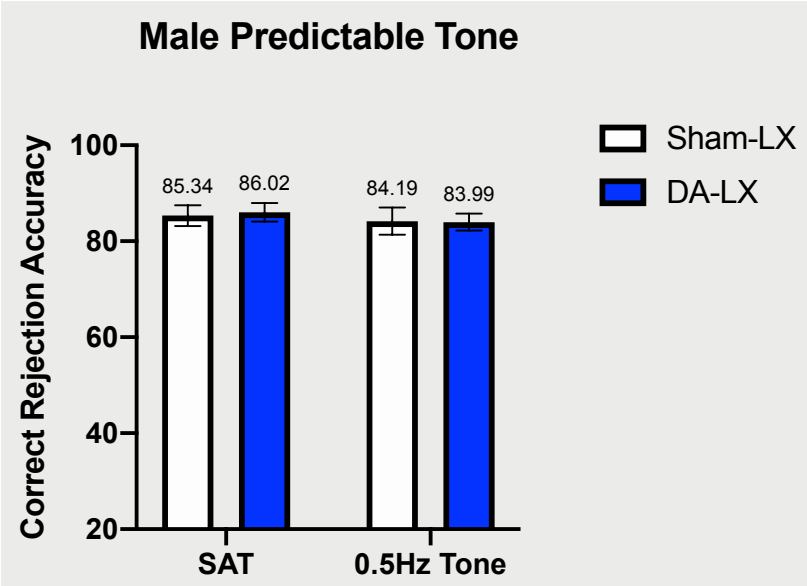

Figure 3.18: Neither Sham-LX males (white bars, n = 10) or DA-LX males (blue bars, n = 8) showed no change in accuracy on non-signal trials (collapsed across block) when the 0.5Hz tone was present compared to baseline performance. Means are displayed above each bar, error bars are SEMs.

Predictable Pellet Delay Hits Females: Imposing a 2s delay of reward produced an decline in accuracy for signal trials compared to baseline in all females ($F(1, 13) = 10.88$, $p < 0.01$). The 2s delay failed to differentiate performance on signal trials between the lesion groups (all $p > 0.05$). Delaying reward 2 seconds did not increase errors of omission for signal trials ($F(1, 13) = 4.51$, $p = 0.05$; baseline: $M = 0.97 \pm 0.25$: 2s delay: $M = 1.35 \pm 0.27$). The predictable delay did not alter the slight non-signal side bias in females (baseline: $M = 0.36 \pm 0.02$; predictable pellet delay: $M = 0.40 \pm 0.02$.)

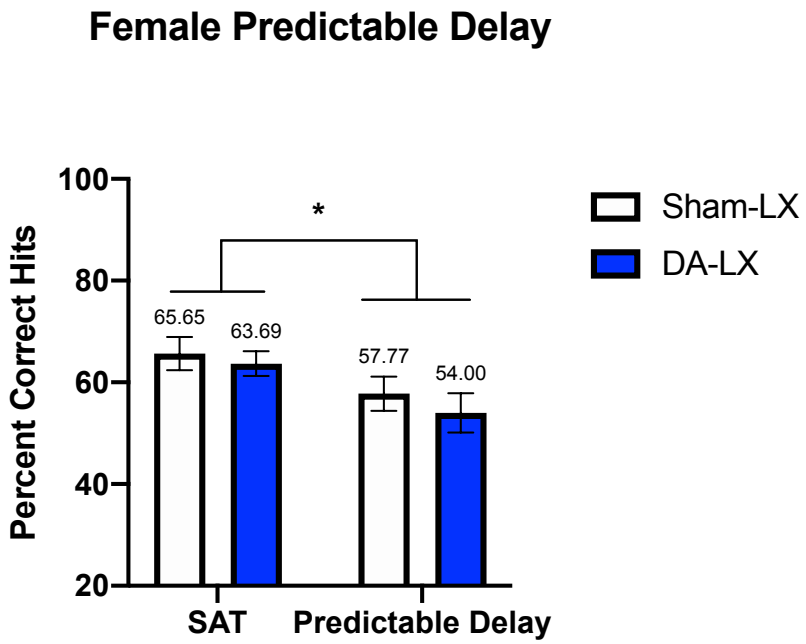

Figure 3.19: Both Sham-LX females (white bars, n = 8) and DA-LX females (blue bars, n = 7) showed a decline in accuracy for signal trials (collapsed across signals and blocks) when a 2s delay was imposed compared to baseline accuracy. Means are displayed above each bar, error bars are SEMs.

Predictable Pellet Delay Correct Rejection Accuracy Females: Unlike for signal trials, imposing a 2s delay of reward did not alter female's accuracy for non-signal trials ($F(1, 13) = 0.71$, $p = 0.42$). Further, the 2s delay failed to differentiate performance on non-signal trials between the lesion groups (all $p > 0.05$).

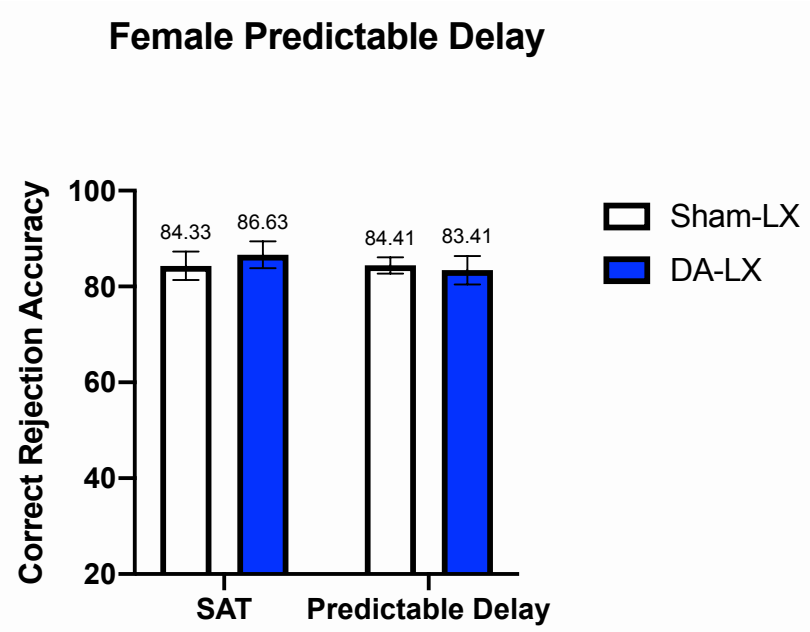

Figure 3.20: Both Sham-LX (white bars, n = 8) and DA-LX (blue bars, n =7) showed no change in accuracy on non-signal trials (collapsed across block) when reward was delayed 2s in compared to baseline. Means are displayed above each bar, error bars are SEMs.

Predictable Pellet Delay Hits Males: Unlike the females, there was no main effect of imposing a 2s delay of reward ($F(1, 16) = 0.01$, $p = 0.92$). However, the effects of imposing a 2s delay of reward interacted with signal length ($F(2, 32) = 3.74$, $p = 0.04$). Where males accuracy for 500msec signal trials when reinforcement was delayed was worse than baseline (baseline vs 2s delay: $t(17) = 2.94$, $p < 0.01$), and 100msec and 25msec signal trials did not differ from baseline

(baseline vs. 2s delay; 100msec: t(17) = -0.25, p = 0.81; 25msec: t(17) = -1.64, p = 0.12) (corrected $\alpha = 0.02$; 3 comparisons). The 2s delay of reward failed to produce any significant difference on the basis of lesion (main effect and interactions with day, block, and signal all p > 0.05). Errors of omission did increase for signal trials when reward was delayed (F(1, 16) = 7.10, p = 0.02; baseline M = 0.14 ± 4.40; 2s delay: M = 0.40 ± 0.11), however, omissions did not differ by lesion, block, or signal length (all p > 0.05)). Predictable pellet delay did not incur a side bias in male subjects. However there was a day by block interaction (F(2, 32) = 3.86, p = 0.03) and further analysis revealed that the predictable delay decreased the slight non-signal side bias in block 2 seen at baseline (baseline v. predictable delay; b1: t(17) = 0.08, p = 0.94; b2: t(17) = 4.47, p < 0.01; b3: t(17) = 0.16, p = 0.88).

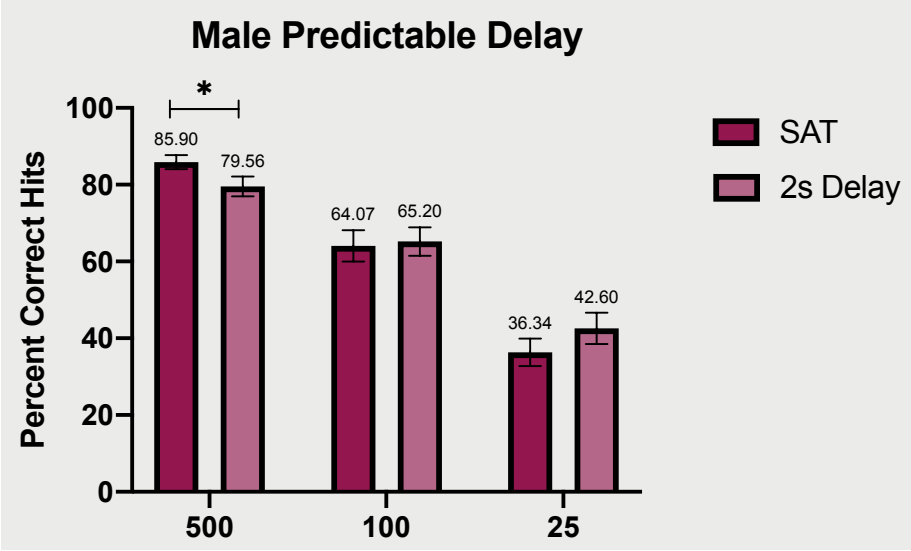

Figure 3.21: When reward was delayed 2s (maroon bars, n = 18) all males showed only a decline in 500msec hits accuracy, but not 100msec or 25msec hits compared to baseline performance (light maroon bars, n = 18). Means are displayed above each bar, error bars are SEMs, * indicates p < 0.02.

Predictable Pellet Delay Correct Rejection Accuracy Males: Unlike for females, when a 2s delay was imposed, subjects accuracy on non-signal trials declined ($F(1, 16) = 7.58$, $p = 0.01$). This interacted with dopamine lesions ($F(1, 16) = 4.88$, $p = 0.04$), and further analysis revealed that males with DA-LX were insensitive to the delay (baseline vs. 2s delay: $t(7) = 0.54$, $p = 0.61$) but males with Sham-LX were not (baseline vs. 2s delay: $t(9) = 3.12$, $p = 0.01$) (corrected $\alpha = 0.03$; 2 comparisons). Imposing a 2 second delay in reward did not significantly increase errors of omission on non-signal trials ($F(1, 16) = 0.07$, $p = 0.79$; baseline: $M = 0.14 \pm 0.04$; 2s delay: $M = 0.40 \pm 0.11$).

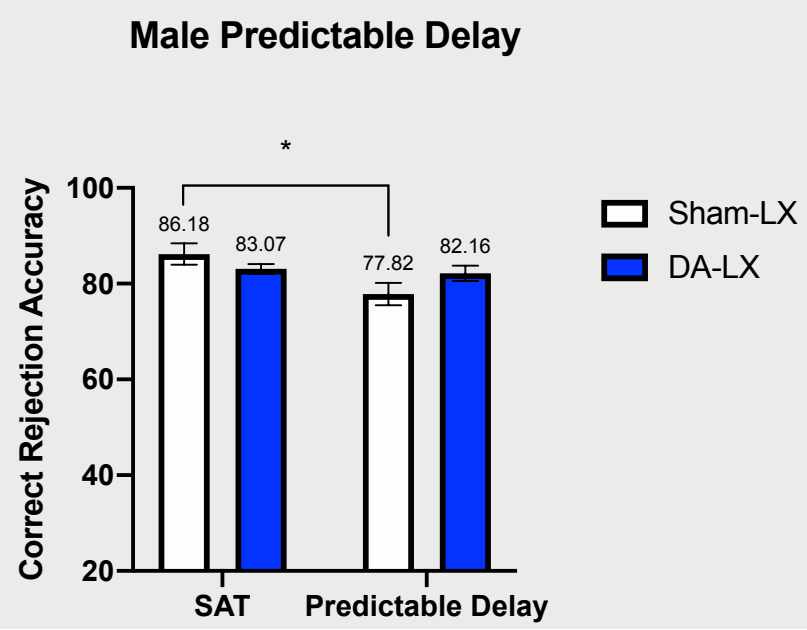

Figure 3.22: Males with Sham-LX (white bars, n = 10) showed a decline in accuracy for non-signal trials (collapsed across blocks and signal lengths) when a 2s delay was imposed compared to baseline performance. Males with DA-LX (blue bars, n = 8) show preserved correct rejection accuracy when reward was delayed 2 seconds. Means are displayed above each bar, error bars are SEMs, * indicates $p < 0.03$.

Unpredictable Pellet Delay Hits Accuracy Females: Similar to when a 2s delay of reward was imposed, when reward was delayed unpredictably, females accuracy on signal trials decreased (day: $F(1, 13) = 5.27$, $p = 0.04$). However, unlike when the 2s delay was imposed, the effects of the unpredictable delay interacted with time on task and dopamine lesions ($F(2, 26) = 3.84$, $p = 0.04$). When assessed, females with sham-LX showed a decrease in accuracy on signal trials compared to baseline over time on task (baseline vs. unpredictable delay; block 1: $t(7) = 0.41$, $p = 0.69$; block 2: $t(7) = 1.25$, $p = 0.25$; block 3: $t(7) = 2.91$, $p = 0.02$), however, this did not hold up to the Bonferroni correction (corrected $\alpha < 0.01$; 6 comparisons). DA-LX females were unaffected by the unpredictable delay (baseline vs. unpredictable delay; block 1: $t(6) = 1.49$, $p = 0.19$; block 2: $t(6) = 1.48$, $p = 0.19$; block 3 $t(6) = -0.04$, $p = 0.97$).

The unpredictable delay of reward did not increase errors of omission ($F(1, 13) = 2.51$, $p = 0.14$; baseline: $M = 0.79 \pm 0.17$; unpredictable delay: $M = 1.00 \pm 0.18$) or side bias (Sham-LX: $M = 0.38$; DA-LX: $M = 0.41$). When the presence of a side bias was analyzed, there was an interaction of day x block x lesion ($F(2, 26) = 4.15$, $p = 0.03$). Further analysis revealed that Sham-LX females showed a more significant non-signal lever bias than DA-LX females in block 2 when reinforcement was delayed unpredictably (Sham-LX vs. DA-LX; baseline b2: $t(13) = 2.42$, $p = 0.03$), however, this did not hold up to the corrected $a < 0.01$; 6 comparisons)

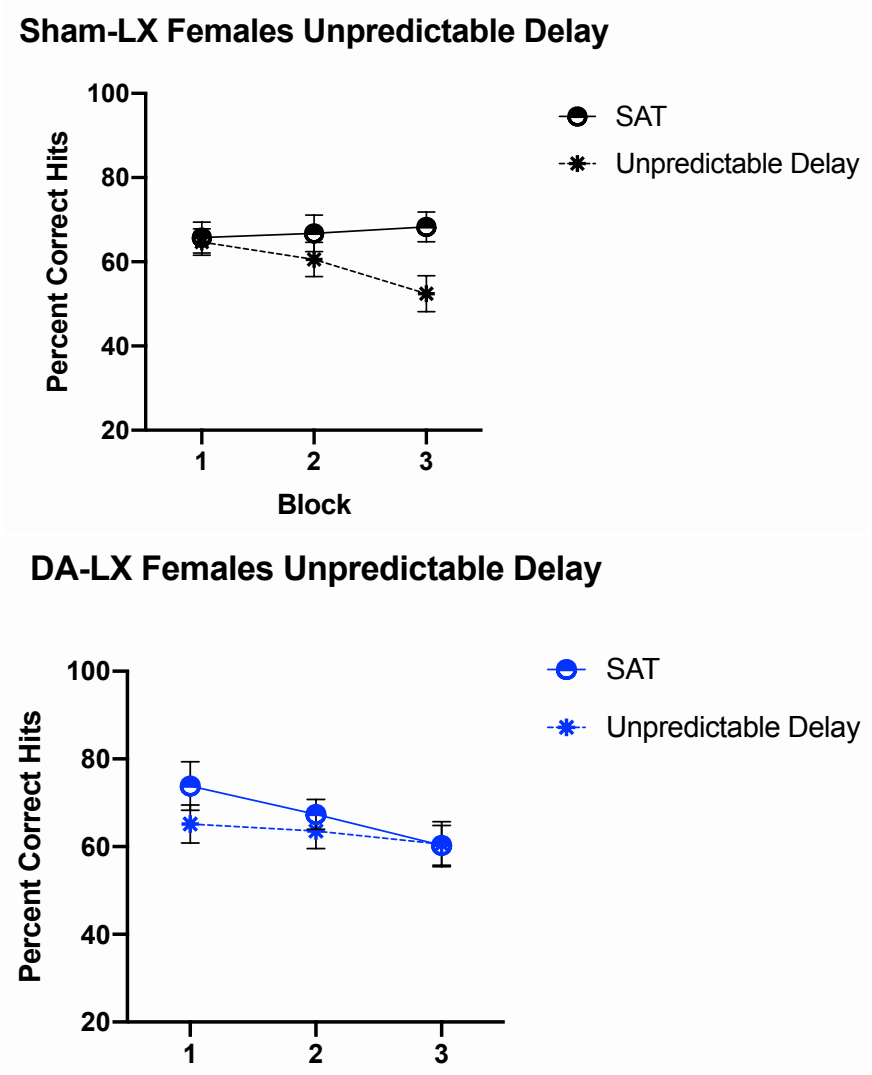

Figure 3.23: Sham-LX females (top, n = 8) and DA-LX females (bottom, n = 7) show the same accuracy for signal trials over time on task when reinforcement was delayed unpredictably (Sham-LX, black star and dashed line; B1: M = 64.70 ± 3.14; B2: M = 60.57 ± 4.10; B3: M = 52.24 ± 4.28; DA-LX, blue star and dashed line; B1: M = 65.18 ± 4.35; B2: M = 63.54 ± 4.00; B3: M = 60.57± 5.13) as baseline (Sham-LX, black half circle, solid line; B1: M = 65.74 ± 3.69; B2: M = 66.77 ± 4.33; B3: 68.33 ± 3.55; DA-LX, blue half circle, solid line; B1: M = 73.81 ± 5.54; B2: M = 67.34 ± 3.42; B3: M = 60.30 ± 4.54). Error bars are SEMs.

Unpredictable Pellet Delay Correct Rejection Accuracy Females: While there was no main effect of imposing an unpredictable delay ($F(1, 13) = 2.62$, $p = 0.13$), the effects of unpredictable

delay interacted with time on task ($F(2, 26) = 4.20$, $p = 0.03$). Further analysis showed that unlike at baseline, and when a 2s delay was imposed, females showed cognitive fatigue for non-signal trials when reward was delayed unpredictably (corrected $\alpha = 0.03$; 2 comparisons; unpredictable delay: block 1 vs. block 2: $t(14) = -0.88$, $p = 0.39$; block 2 vs. block 3: $t(14) = 3.35$, $p < 0.01$). Imposing an unpredictable delay did not differentiate performance between lesion groups (all $p > 0.05$), nor did it increase errors of omission for non-signal trials ($F(1, 13) = 1.59$, $p = 0.23$; baseline: $M = 2.58 \pm 0.53$; unpredictable delay: $M = 3.16 \pm 0.60$).

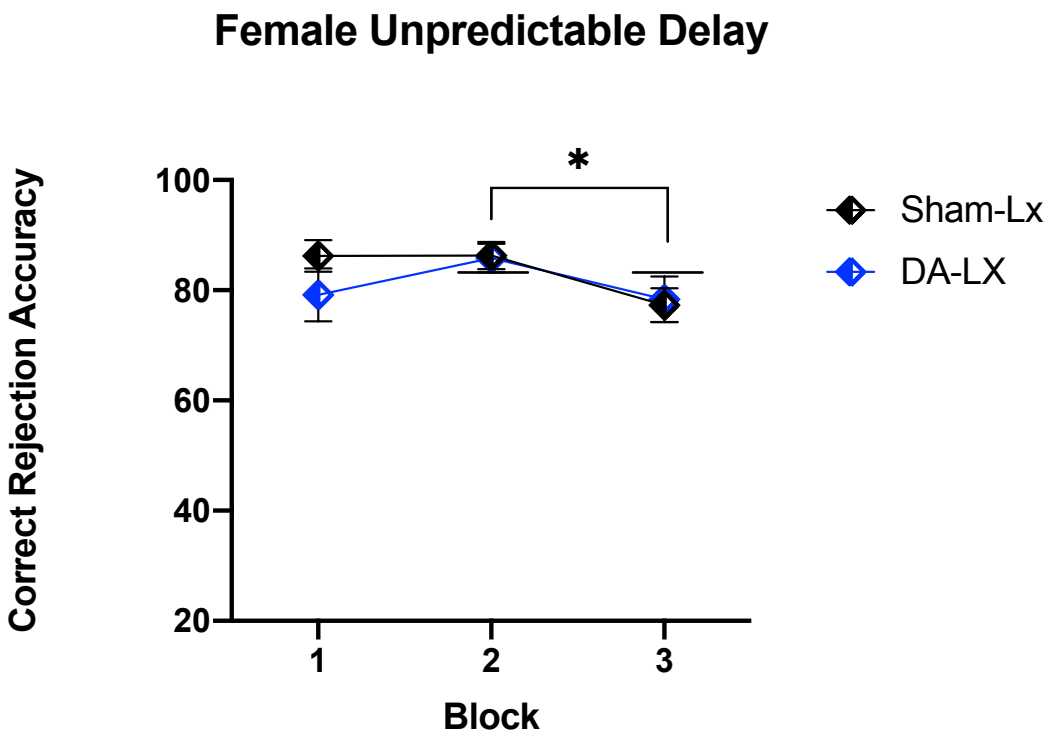

Figure 3.24: All females showed an decrease in accuracy for non-signal trials over time on task when reward was delayed unpredictably (block squares; block 1: $M = 82.93 \pm 2.79$; block 2: $M = 86.08 \pm 1.74$; block 3: $M = 77.81 \pm 2.46$; $n = 15$) compared to baseline (black circles; block 1: $M = 87.40 \pm 2.00$; block 2: $M = 81.95 \pm 2.85$; block 3: $M = 85.45 \pm 3.30$; $n = 15$)

Unpredictable Pellet Delay Hits Accuracy Males: Unlike in female subjects, The unpredictable delay of reward did not significantly impact hits accuracy in males ($F(1, 16) = 1.14$, $p = 0.30$), furthermore day did not interact with block, signal, and lesion all $p > 0.05$. The unpredictable delay of reward did not dissociate the two lesion groups (all $p > 0.05$).

Unpredictable Pellet Delay Correct Rejection Accuracy Males: Similar to signal trials, unpredictable delay of reward did not impact males accuracy for non-signal trials ($F(1, 16) = 0.22$, $p = 0.65$), furthermore day did not interact with block, signal, and lesion all $p > 0.05$. The unpredictable delay of reward did not dissociate the two lesion groups (all $p > 0.05$).

Removal of Reward Hits Accuracy Females: All females accuracy on signal trials increased when reward was removed ($F(1, 13) = 5.19$, $p = 0.04$). The effects of removing reward interacted with time on task ($F(2, 26) = 15.51$, $p < 0.01$). In the initial block of trials, females showed an increase in accuracy on signal trials compare to baseline performance when reward was removed (corrected $\alpha = 0.02$; 3 comparisons; baseline vs. removal of reward: block 1: $t(14) = -8.05$, $p < 0.01$) however, this improvement was not maintained over time on task (baseline vs. removal of reward; block 2: $t(14) = -2.00$, $p = 0.07$; block 3: $t(14) = -0.77$, $p = 0.46$). Unlike the unpredictable delay, removal of reward failed to produce differences in performance between the lesion groups (all $p > 0.05$). Removing reward did increase omissions in all females ($F(1, 13) = 29.38$, $p < 0.01$; baseline: $M = 0.72 \pm 0.17$; removal of reward: $M = 1.65 \pm 0.21$), however there is no interactions with lesion or block (all $p > 0.05$). Removal of reinforcement did not create a side bias in females ($F(1, 13) = 0.42$, $p = 0.53$) Baseline $M = 0.42 \pm 0.02$, removal of reinforcement $M = 0.43 \pm 0.02$.

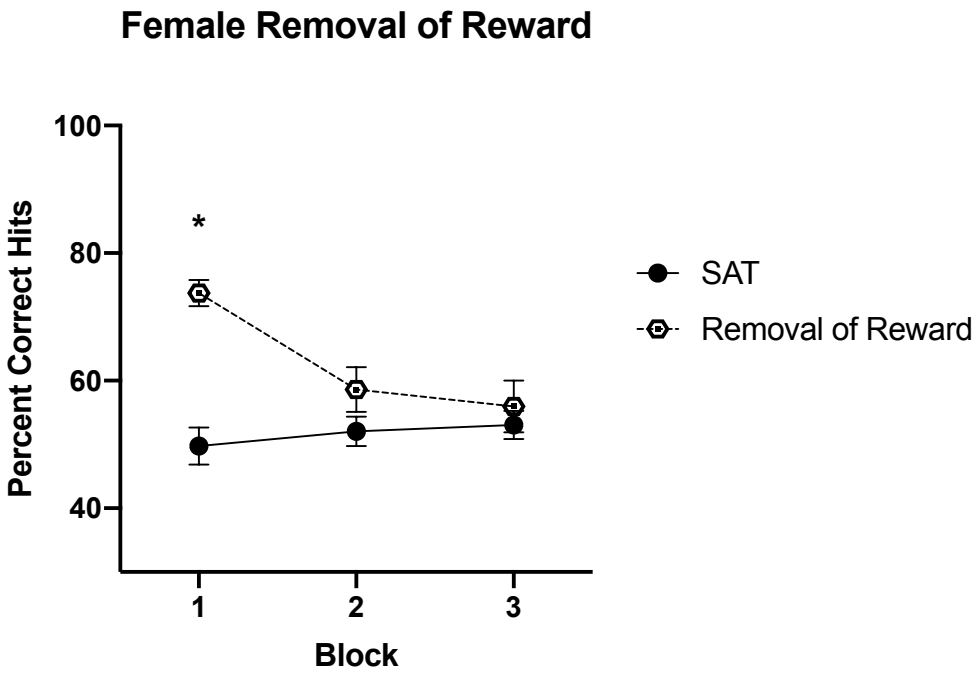

Figure 3.25: When reward was removed, all females showed an initial increase in signal trial accuracy (combined across all signal lengths; black square; block 1: M = 73.75 ± 2.05) compared to baseline performance (black circle; block 1: M = 49.77 ± 2.94). However, this increase returned to baseline performance over time on task (block 2: baseline: M = 52.06 ± 2.31; removal of reward: M = 58.61 ± 3.51and block 3 baseline: M = 53.04 ± 2.21; removal of reward: M = 55.95 ± 4.05). Error bars are SEMs.

Removal of Reward Correct Rejection Accuracy Females: While there was a main effect of day ($F(1, 13) = 5.74$, $p = 0.03$) the demands of removing reward did not interact with lesion ($F(1, 13) = 0.56$, $p = 0.47$). However, unlike baseline or any other variant, overall, dopamine lesions showed worse performance ($F(1, 13) = 5.98$, $p = 0.03$).

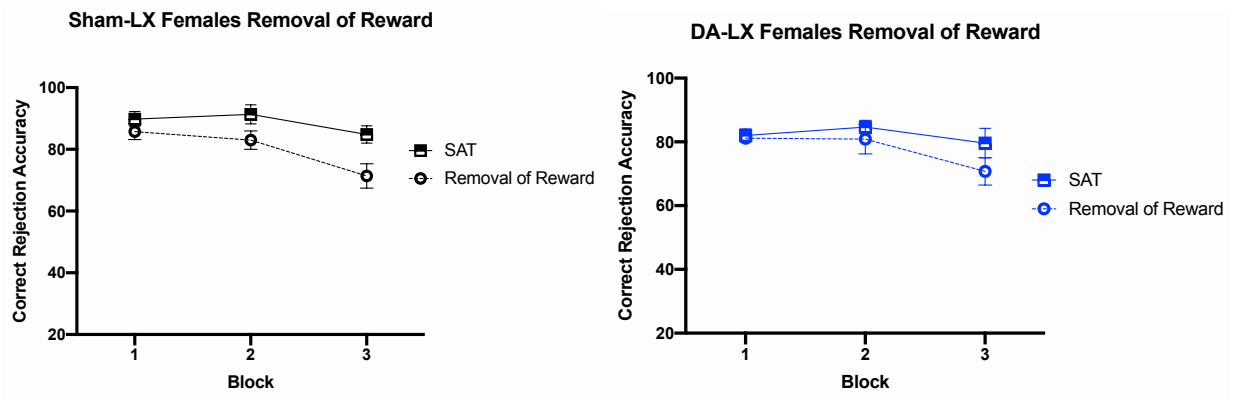

Figure 3.26: Sham-LX females showed a decrease in accuracy for non-signal trials when reward was removed (black open circles with dashed line; B1: M = 85.75 ± 2.59; B2: M = 3.01; B3: M = 71.39 ± 3.93) compared to baseline performance (black half open square, solid line; B1: M = 89.78 ± 2.49; B2: M = 91.35 ± 3.09; B3: M = 84.84 ± 2.81). DA-LX females also showed a decrease in accuracy for non-signal trials when reward was removed (blue open circles with dashed line; B1: M = 81.15 ± 1.70; B2: M = 80.90 ± 4.67; B3: M = 70.75 ± 4.31) compared to baseline performance (blue half open square, solid line; B1:M = 82.02 ± 2.05; B2: M = 84.67 ± 2.16; B3: M = 79.64 ± 4.67). Error bars are SEMs.

Removal of Reward Hits Accuracy Males: In contrast to the females, there was no main effect of day ($F(1, 16) = 0.59$, $p = 0.46$). However the effects of removing reward interacted with signal length ($F(2, 32) = 24.36$, $p < 0.01$). Further analysis (corrected $\alpha = 0.02$; 3 comparisons) revealed that all males accuracy on 500msec signal trials declined compared to baseline when reward was removed (baseline vs. removal of reward: $t(17) = 5.90$, $p < 0.01$). Further, males accuracy on 100msec and 25msec signal trials was preserved when reward was removed (baseline vs. removal of reward; 100msec: $t(17) = 0.93$, $p = 0.37$; 25msec: $t(17) = -2.74$, $p = 0.02$). While all

males with DA-LX performance was worse overall ($F(1, 15) = 4.77$, $p = 0.04$) this failed to interact with the effects of removing reward (all $p > 0.05$). When reward was removed, all males showed an increase in errors of omission during signal trials ($F(1, 16) = 27.76$, $p < 0.01$).The number of errors of omission were not significantly different by lesion when reward was removed (all $p > 0.05$; DA-LX baseline: $M = 0.25 \pm 0.09$; removal of reward: $M = 1.07 \pm 0.31$; Sham-LX baseline: $M = 0.08 \pm 0.08$; removal of reward: $M = 1.48 \pm 0.28$). All males showed a correction of their slight non-signal lever side bias when reinforcement was removed ($F(1, 16) = 4.78$, $p = 0.04$). Baseline $M = 0.41 \pm 0.02$ removal of reinforcement $M = 0.44 \pm 0.02$. There was no difference in side-bias between the lesion groups (all $p > 0.05$).

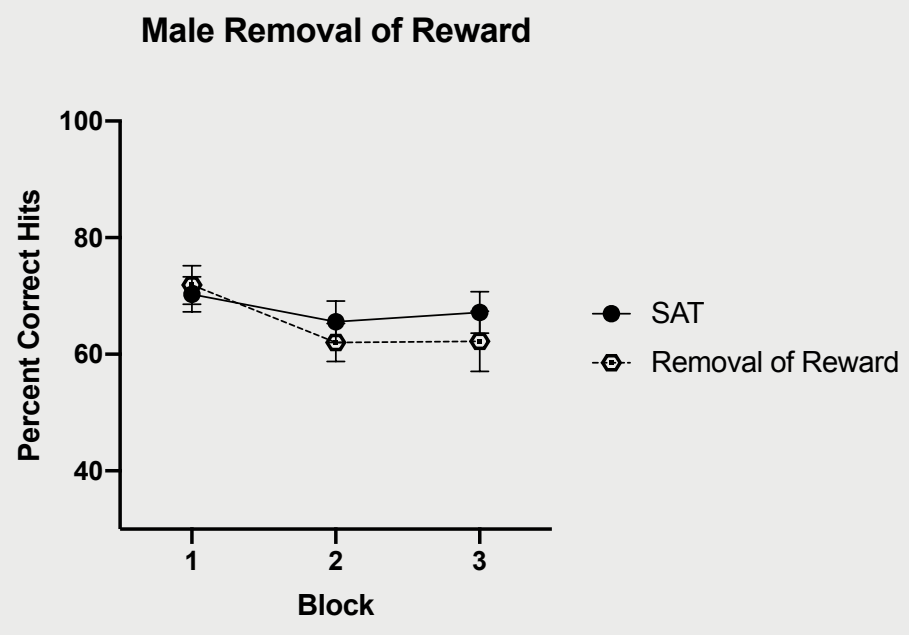

Figure 3.27: All males showed no change in accuracy for signal trials over time on task (collapsed across signal lengths) when reinforcement was removed (open polygon, dashed lines, n = 18; B1: $M = 71.90 \pm 3.33$; B2: $M = 62.20 \pm 3.35$; B3: $M = 62.29 \pm 5.20$) compared to baseline accuracy for signal trials (closed circle, solid lines, n = 18; B1: $M = 70.30 \pm 3.30$; B2: $M = 65.61 \pm 3.64$; B3: $M = 67.24 \pm 3.67$). Error bars are SEMs.

Removal of Reward Correct Rejection Accuracy Males: Contrasting females performance**,** removal of reward produced a decline in non-signal trial accuracy in male subjects (day $F(1, 16) = 21.06$, $p < 0.01$). However, the effects of removing reward interacted with time on task and lesion (day x block x lesion: $F(2, 32) = 3.82$, $p = 0.03$). Further analysis (corrected $\alpha < 0.01$; 6 comparisons) revealed that males with Sham-LX accuracy on non-signal trials was worse than baseline in later stages of the task (baseline vs. removal; block 1: $t(9) = 0.41$, $p = 0.70$; block 2: $t(9) = 3.32$, $p = 0.01$; block 3: $t(9) = 3.80$, $p < 0.01$). However, subjects with DA-LX did not show any difference from baseline performance across time on the task (baseline vs. removal; block 1: $t(7) = 1.11$, $p = 0.30$; block 2: $t(7) = 2.26$, $p = 0.06$; block 3: $t(7) = -0.34$, $p = 0.74$). Errors of omission on non-signal trials increased when reward was delayed ($F(1, 16) = 49.03$, $p < 0.01$; baseline: $M = 0.52 \pm 0.17$; removal of reward: $M = 4.20 \pm 0.56$) however, omissions did not differ on the basis of lesion (all $p > 0.05$).

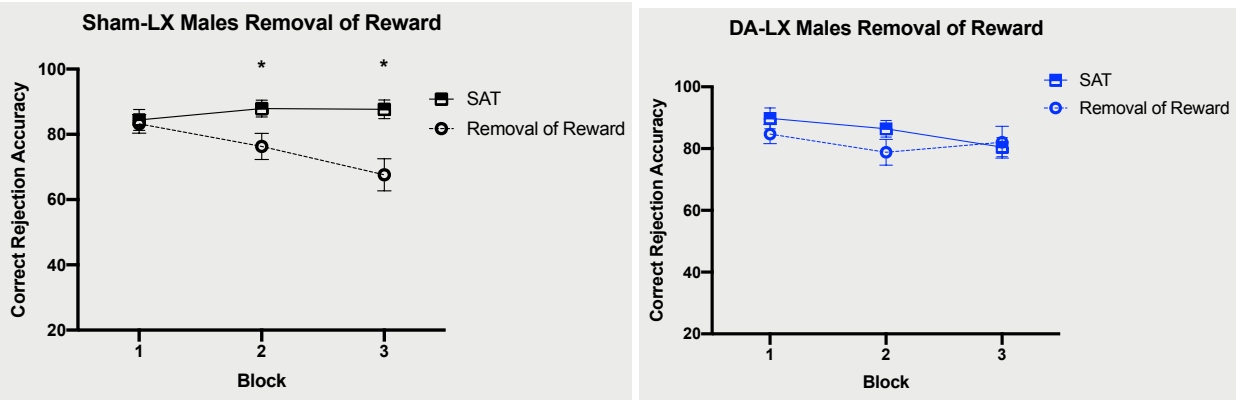

Figure 3.28: Males with sham-LX (left) show a decline in non-signal trial accuracy when reward was removed (black open circle and dashed line; B1: $M = 83.20 \pm 2.99$; B2: $M = 76.38 \pm 4.00$; B3: $M = 67.67 \pm 4.96$) compared to baseline (black half square and solid line; B1: $M = 84.48 \pm 3.22$; B2: $M = 87.90 \pm 2.65$; B3: $M = 87.71 \pm 2.95$), showing an decline in accuracy for non-signal trials over time on task. Males with DA-LX (right) show no decline in correct rejection accuracy when reward was removed (blue open circle and dashed line; B1: $M = 84.70 \pm 3.10$; B2: $M = 78.81 \pm 4.23$; B3: $M = 82.16 \pm 5.24$) compared to baseline (blue half square and solid line; B1: $M = 89.82, \pm 3.44$; B2: $M = 86.45 \pm 2.70$; B3: $M = 80.55 \pm 3.14$).

Discussion

In the baseline SAT, all subjects showed an increase in accuracy for signal trials and non-signal trials with repeated testing sessions. There was a difference in the SAT over time on task. When the data were collapsed across the five baseline SAT sessions, DA-LX females, but not DA-LX males or Sham-LX males, showed a loss of signal-dependent performance over time on task, specifically in block 3. The decline in signal-dependent performance in block 3 for DA-LX females reflects a loss of the ability to detect the more salient signals with time on task. Detriment at the baseline task has been implicated as a deficit in bottom-up, signal driven processes, where signals do not activate the cholinergic system in the PPC (Sarter, Hasselmo, Bruno, & Givens, 2005). As this deficit only occurred over time on task (block 3), it may indicate a decline with the increase in cognitive load over time on task.

In the sustained attention task, distractor sessions are used to increase top-down control. Specifically, the increase in top-down control seen when subjects encounter the 0.5Hz light has been attributed to cholinergic input from the mPFC (Gill et al., 2000). The ability to filter cross-modal distractors has been attributed to connections from the PPC to PFC, as has been seen when cholinergic lesions of the PFC produced an inability to filter the 0.5hz tone (Newman & McGaughy, 2008b). In the present study, all subjects showed a decline in accuracy for signal trials when the flashing houselight was present. Sham-LX, but not DA-LX subjects showed a decline in accuracy for 25msec signals in the presence of the flashing houselight. This likely reflects a floor effect in the DA-LX females' ability to report the 25msec signal trials while they are embedded in a dynamic stimulus range. This is likely not a perceptual deficit but an attentional one as eliminating the dynamic stimulus range decreases the deficit in 25msec signal detection (Newman & McGaughy, 2008b). However, this difference failed to remain significant

when corrected for multiple comparisons. All males showed a decline in signal trial accuracy when the flashing houselight was present, but this did not differ between the lesion groups.

In the baseline session preceding the 0.5Hz tone session, Sham-LX females and DA-LX females did show a difference in accuracy for signal trials. In the 0.5Hz tone session, however, there was no difference in accuracy for signal trials between the Sham-LX females and DA-LX females. All males showed the ability to filter the 0.5Hz tone, and there was no difference in baseline or 0.5Hz tone session accuracy for signal trials between the lesion groups.

The lack of difference between the Sham-LX and DA-LX subjects in the distractor sessions mirrors data seen with IBO-LX to the ACC (Newman & McGaughy, 2011). The distractors used in the SAT are novel and have never been paired with a reinforcement history before. While the distractors require an increase in top-down control, this has been shown to be linked to a cholinergic loop (Gill et al., 2000; Newman & McGaughy, 2008b; Sarter, Gehring, et al., 2005; Sarter et al., 2001), and does not appear to be impacted by dopaminergic lesions to the ACC. Further, the lack of differences between the lesion groups in both sexes in the presence of novel distractors suggests that DA-LX does not increase susceptibility to all kinds of distracting stimuli and that the deficits seen in the CD of the ASST may be due to the previous reinforcement history associated with the complex dimensions which are introduced at that stage.

The ability to update reinforcement contingencies without the presence of distractors was explicitly tested. All females showed an inability to update reinforcement contingencies when a two-second delay was imposed, as seen by the decline in accuracy for signal trials. When reinforcement is delayed, subjects still need to maintain the response rule as they did at baseline and incorporate the information about the delay not as an error cue, but as a new expected reinforcement time. With the consistent delay, females appear to be altering behavior as if they

were experiencing an error. Males showed no difference in signal trial accuracy when reinforcement was delayed two seconds but did show impaired performance on non-signal trials. Analysis of the side bias data suggests this sex difference may represent sex-related changes in response strategy but requires further assessment.

Dopamine in the ACC is thought to be responsible for the detection of errors (Holroyd & Coles, 2002; Hyman et al., 2017; Hyman et al., 2012). From the HRL-ACC framework, the error signals generated by dopamine in the ACC are integrated into the model when a shift in action is needed (Holroyd & Umemoto, 2016). One of these signals is the feedback-related negativity (FRN), which has been seen in rodents (Warren et al., 2015). The FRN is hypothesized to initiate the shift in behavior needed following a discrepancy in error prediction (Holroyd & Umemoto, 2016). Feedback delay has been shown to impact the FRN generated by the ACC, decreasing it, and inhibiting behavioral modification from negative error feedback (Höltje & Mecklinger, 2018). In the HRL-ACC framework, the FRN is the cue generated by the ACC to recruit the DLPFC to either inhibit or disinhibit motor neurons caused by dopaminergic reward positivity error signals (RPEs) (Holroyd & Umemoto, 2016).

In the unpredictable delay condition, subjects received reinforcement immediately following hits and correct rejections one-third of the time. This may have been sufficient to prevent the decline in accuracy for signal trials in female subjects as the female rodents showed an ability to adjust to the unpredictable delay in reinforcement for signal trials. Sham-LX, but not DA-LX females showed a decrease in accuracy for signal trials in block 3, however, this did not remain significant when adjusted for multiple comparisons. When reinforcement was delayed unpredictably, all males showed an ability to adjust for signal trials and non-signal trials. In the removal of reinforcement condition, females initially showed improved signal accuracy

compared to baseline and no differences in accuracy for non-signal trials. Sham-LX but not DA-LX males show decreased non-signal performance with prolonged time on task when reinforcement is removed. This data suggest that DA-LX females adapt more readily in these conditions than do DA-LX males.

When reward is removed altogether, dopamine signals in the ventral tegmental area (VTA) decline past baseline tonic firing rates (Schultz, 1997). This decline in firing does not persist over time, and with the continued omission of reinforcement, VTA dopamine signaling returns to baseline levels (Hollerman & Schultz, 1998; Schultz, 1997). When reinforcement is delayed, dopamine firing increases at the new reinforcing delivery time and eventually returns to baseline firing, having adjusted (Hollerman & Schultz, 1998). DA-LX male rats were less sensitive than SHAM-LX male rats to changes in reinforcement contingencies, but this was not found in female subjects. The delay and lack of adjustment may indicate an increased sensitivity to errors that is sex-specific after dopamine lesions to the ACC. Human males have been shown to have larger and longer error-related negativity following an error compared to females (Larson, South, & Clayson, 2011; Omura & Kusumoto, 2015). Males may require more time to integrate error signals, which is why the current male subjects were able to update at 2s, where females subjects were not.

Together the SAT and variant sessions show that dopaminergic lesions in the ACC do not produce a global decrease in accuracy for signal trials with time on task, increase susceptibility to novel distractors, nor a global inability to update reinforcement contingencies. Unlike in the IBO-LX study, DA-LX subjects did not show a baseline decrease in signal trial accuracy over time on task (block 2) (Newman & McGaughy, 2011). This suggests that another neuromodulatory system that was depleted following the IBO-LX may have been responsible for

the decreased accuracy over time on task. DA-LX subjects were no more impaired in reporting signals when in the presence of the 0.5Hz light and tone compared to Sham-LX subjects, supporting the previous findings that the ACC is not necessary to filter rewards that do not have a prior reinforcement history (Newman & McGaughy, 2011).

The ability to update reinforcement contingencies was not globally inhibited in DA-LX subjects when no distracting stimuli were present. Sham-LX and DA-LX males were able to adjust to all alterations in reinforcement timing and maintained accuracy for signal trials but were impaired in processing non-signal trials. Sham-LX and DA-LX females showed an inability to adjust to the predictable delay but were able to adjust with time when reinforcement was removed and delayed unpredictably. How the data from the sustained attention task can be used to explain the data, and deficits seen in DA-LX on the ASST will be discussed in chapter 5.

CHAPTER IV

HISTOLOGICAL ASSESSMENT OF LESIONS

Tyrosine Hydroxylase Procedure

Animals were exsanguinated with 0.9% saline, followed by 4% paraformaldehyde in phosphate buffer. Brains were removed and placed in a 30% sucrose solution until they sunk and were then sliced. Coronal sections were sliced at 40 microns where every fifth slice of tissue was separated to create five complete sets from each subject, as seen in (Newman & McGaughy, 2008b). One set was sliced into tris buffered saline (TBS) and four were sliced into a 15% glycerol solution and frozen in an -80 C freezer (Stirling Ultracold, Athens, OH). Alternating sets of tissue allow for multiple histological assessments to be conducted. Brains were stained for tyrosine hydroxylase, briefly, sections were rinsed in TBS (three times for 10 min), before entering into a bath of methanolic peroxide (90ml TBS; 10ml 100% MeOH; 2ml 30% H2O2) for 15min, followed by three more rinses of TBS, transferred into 40% blocking solution of TBS+ normal horse serum (vector laboratories, S-2000) for 30 min, and an overnight incubation in primary antibody (mouse anti-TH; 1:5000 dilution in TBS+; ImmunoStar, 22941). Three rinses of TBS (10 min each) and the 1.5 h in secondary antibody (biotinylated donkey anti-mouse; 1:1000 dilution in TBS+; Jackson Immunoresearch, 716-065-150). Three rinses of TBS (10 min each) and then 1 h in tertiary incubation (horseradish peroxidase-conjugated streptavidin 1:1600 dillutoin in TBS with Triton X-100; Jackson Immunoresearch, 016-030-084). Following four rinses in TBS (10min each) sections were placed into a bath of diaminobenzidine and nickel

cobalt solution, 30 microl of 3% hydrogen peroxide were added to begin reaction. Slices were quenched in three rinses of TBS once fibers became visible (3-7min). Sections were mounted with gelatin on gelatin coated slides. Histological assessment of fiber loss was made using a modified grid counting procedure (McGaughy et al., 1996; Ross, McGaughy, & Eichenbaum, 2005). Sections were assessed on an Olympus Optical BX51 microscope (Optical Analysis Corp.) using the 40X objective. Images of the sections were photographed using an Amscope microscope camera (Amscope, Irvine, CA). Images were sized to 2048 x 2048 before being opened in SPOT basic version 5.0.27 (Diagnostic Instruments, Sterling Heights, MI) and having a 300x300 mm grid applied. The number of fibers which crossed the perimeter of the grid were counted. Images were taken from both hemispheres at the injection site locations in the anterior cingulate cortex (from bregam +2.7 and +2.2).

Statistical Analysis

The extent of the damage at injection sites was assessed using a mixed factors ANOVA with hemisphere (2 levels) and position (2 levels; +2.7. +2.2) as within subjects' factors and a between subjects factor of Lesion (2 levels).

Results

Statistical analysis showed that loss was centered around the injection site locations (+2.7 and +2.2) Lesion: $F(1, 23) = 172.93$, $p < 0.01$, but did not differ by hemisphere, location, or sex (rostral caudal: $F(1, 23) = 0.94$, $p = 0.34$; hemisphere: $F(1, 23) = 1.76$, $p = 0.20$; Sex: $F(1, 23) = 1.18$, $p = 0.29$;). Lesioned animals showed a 53.6% loss of TH positive fibers in area ACC of +2.7 and +2.2.

CHAPTER V

GENERAL DISCUSSION

One limitation of the current study is the use of only tyrosine hydroxylase (TH) staining to assess deafferentation. TH is the first step in the catecholamine synthesis pathway and as such, staining for TH would not stain exclusively for dopamine, but it will also stain for norepinephrine (NE). Due to the inability to access university space during the COVID-19 pandemic, staining for dopamine beta-hydroxylase, and further examination of TH stained tissue could not be completed by the graduate school's thesis completion deadline. Dopamine Beta Hydroxylase (DBH) is a NE specific marker and would allow for a more complete assessment of the damage caused by the anti-DAT saporin (Newman & McGaughy, 2008a). As DBH stains for NE, and TH stains for both DA and NE, we expect lower DBH fiber counts, with the difference between the DBH and TH, counts confirming the DA specific loss in these subjects. In addition, lesion assessment was limited to just the injection target location. Only confirming that the lesion depleted dopamine in the target region (ACC) does not show that there was no damage to other neighboring regions, each of which has been shown to have functional differences. Further assessment of the damage caused by the anti-DAT saporin will extend 1mm rostral and 1mm caudal to the lesion site. If the damage is seen at these boundaries, imaging will continue to 0.5mm beyond the end of the loss. At each of these locations, images will be taken from ACC as well as IL and PL in both hemispheres totaling a minimum of 24 images per subject (Newman & McGaughy, 2008a, 2008b). Because public health concerns prohibit the collection of these data,

the discussion is based on the available data showing there is a loss to the dopamine system with the assumption that the toxin has been specific for dopaminergic corticopetal system. This will be confirmed before the submission of these data for peer-review.

Dopamine lesioned subjects did not show the ability to form an attentional set. This can be seen in three crucial ways. First, DA-LX subjects show an increase in trials to criterion at the ID and ED stages of the ASST compared to Sham-LX subjects. Second, Sham-LX subjects, but not DA-LX subjects, show a decrease in trials to criterion at the ID stage compared to the CD stage, indicating that sham-LX subjects were able to apply the attentional set to the novel stimuli, where DA-LX subjects were not. Third, Sham-LX subjects, but not DA-LX subjects, show an increase in trials to criterion at the ED stage compared to the ID stage, showing the cost of having formed an attentional set. Previous data have shown that subjects with ibotenic acid lesions to the ACC can form an attentional set (Newman & McGaughy, 2011). However, there are some differences between the initial study and the present study that may be able to explain this apparent difference.

In the previous study, lesioned subjects showed a severely increased susceptibility to distraction, requiring 86.14±5.39 trials in the CD and CDR before having to use the attentional set in the ID (Newman & McGaughy, 2011). In the present study, while subjects were more impaired compared to Sham-LX subjects, they only required 43.40±2.06 trials in the CD and CDR. Other research has shown that ACC-LX subjects did not form an attentional set and showed increased trials to criterion in the ID compared to Sham-LX subjects (Ng, 2007). One of the previous hypotheses regarding the differences in the ACC-LX studies was due to the lack of CDR stage in Ng and colleges (2007) ASST, and the increase in trials required to meet criterion seen in the CD and CDR of the Newman & McGaughy (2011) study. The Newman &

McGaughy (2011) IBO-LX subjects may have benefited from the extensive number of trials, and therefore experience, in the CD and CDR stages. The extensive experience was not seen in the Ng (2007) ACC-LX subjects due to the lack of trials encompassed by the omission of the CDR stage, or the DA-LX subjects due to fewer trials to criterion in the CD and CDR stages. One way to assess this in the future could be to test DA-LX subjects in a 4ID set shifting task. The task uses the same complex stimuli as were used in the present study, however, following the CD there is no reversal and subjects instead encounter ID1, ID2, ID3, and ID4 before having to shift an attentional set in ED (Alexander et al., 2012). If the DA-LX subjects are capable of forming an attentional set like the IBO-LX subjects were, the additional trials in the set before it needs to be shifted in the 4ID task should allow the DA-LX subjects to form and shift an attentional set as the high trials in the CD and CDR did for the IBO-LX subjects in the 7-stage task.

The present data suggest that dopamine in the ACC is necessary to filter distractors only when current and prior reinforcement feedback is needed to discriminate relevant, and irrelevant attributes of complex stimuli. In the ASST, subjects with DA-LX showed an ability to perform conditional discriminations in all exemplar stages, as well as in the SD. When stimuli became complex, DA-LX subjects showed increased susceptibility to distraction compared to Sham-LX subjects from the addition of the irrelevant dimensions. Data from both the ASST and SAT indicate that this particular distractibility is not to novel stimuli, but rather to the specific inability to ignore distractors with a prior reinforcement history. In the ASST, DA-LX subjects showed deficits on stages where no novel stimuli were introduced (reversal stages), as well as no difficulty when a consistent dimension was changed (LI). The lack of sensitivity to the test of learned irrelevance also indicates that subjects were particularly susceptible to changes in the varying irrelevant dimension in the complex stimuli, as opposed to the consistent never relevant

dimension. In the sustained attention task, DA-LX subjects were no more impacted by the novel distractors than Sham-LX subjects. Together, the data indicate that dopamine depletion in the ACC does not increase susceptibility to distraction from novel stimuli, but rather from variable dimensions of complex stimuli which have a prior reinforcement history.

The idea that dopaminergic lesions to the ACC produced deficits in the ability to ignore variable dimensions of a complex stimulus with a prior reinforcement history adhere to the HRL-ACC framework in conjunction with the idea that dopamine is a stability mechanism. The HRL-ACC framework would suggest that the increased susceptibility to distraction comes from a lack of option selection from the ACC in the face of complex stimuli (Holroyd & Umemoto, 2016). In the stages of the ASST which require simple discriminations subjects do not have to selectively attend to a dimension while ignoring distracting stimuli. In the acquisition of the SAT, DA-LX subjects are also able to learn the response rule for reporting the presence of signals, and the absence of signals. The HRL-ACC framework would posit that the actor-critic connection is sufficient to make decisions in a situation free from distraction. Where the HRL-ACC framework is lacking, is in the specificity for the distraction seen in the ASST and SAT. The framework suggests that with the lack of dopamine in the ACC, the ACC will not send a signal to the DLPFC that an increase in attentional control is needed and will not adjust the selected target to meet the increased demands in the now more complex task (Holroyd & Yeung, 2012). This would explain a general increased susceptibility to all distractions; however, the present data do not support this completely. Rather, the lack of susceptibility to novel distractors leans more into the idea of dopamine as a stability mechanism (Chudasama & Robbins, 2004; Robbins, 2005).

To achieve success at the CD stage, subjects need to maintain responding to the dimension and attribute which was rewarded in the simple discrimination prior and ignore the

newly added variable dimension, and consistent dimension. As both the relevant and irrelevant variable dimensions have a prior reinforcement history, at the CD stage, either of the variable dimensions "might" be predictive of reward, as is seen by in increased trials at the CD compared to the SD in all subjects. A lack of dopamine in the ACC may prevent, as the HRL-ACC framework suggests a stable selection of which dimension is relevant based on insufficient reinforcement/error feedback. When the distractor had no reinforcement history in the SAT, DA in the ACC was not needed to communicate to the DLPFC what stimuli needed to be filtered, as integration of reinforcement feedback was not needed to filter the novel distractor. When the stimuli had a prior reinforcement history, dopaminergic feedback to the ACC may have been needed to select which dimension was currently associated with reward and recruit the DLPFC to implement specific control for that dimension. Without the stability of which dimension was presently associated with a reinforcer, attention was specifically more susceptible to stimulus dimensions that altered, and 'might' have been predictive of reward, as they had been in previous exemplar stages.

DA-LX subjects showed a deficit at all reversal learning stages compared to Sham-LX subjects. This deficit could be due to an inability to update reinforcement contingencies or an inability to overcome a distracting element of a complex stimulus. If subjects showed an inability to update reinforcement contingencies, it would be expected that in an error analysis, subjects would show repeated returns, not to the attribute which previously elicited reinforcement (CDR; light foam shapes; perseverative errors). An inability to update reinforcement contingencies would show and an inability to cease responding to an element once it no longer predicts reward (Alexander, Tait, & Brown, 2012). If DA-LX subjects showed an inability to overcome distraction from a previously reinforced element of the complex stimuli, an error analysis should

show incorrect responses to multiple stimulus elements with a prior reinforcement history (regressive errors). All subjects showed an improvement in reinforcement reversals with successive stages, indicating that DA-LX subjects do not have a global inability to update reinforcement contingencies. An inability to update reinforcement contingencies would be expected to produce no ability to learn over repeated reinforcement reversals, as has been seen with lesions to the orbitofrontal cortex (Tait & Brown, 2007). Future error analysis may reveal an increased susceptibility to distraction from the previously reinforced elements of the complex stimulus at all reversal stages, showing increased regressive as opposed to perseverative errors in DA-LX subjects.

While an error analysis has not been conducted yet, data from the sustained attention task can shed light on DA-LX subjects' ability to update reinforcement contingencies. In the sustained attention task, DA-LX subjects show an ability to update reinforcement contingencies in some situations, indicating that dopaminergic lesions to the ACC do not produce a global inability to incorporate reinforcement feedback. When reinforcement was delayed predictably (2s), all females showed an inability to adjust to the change in reinforcement feedback. Sham-LX, but not DA-LX male rats showed altered non-signal performance when a two-second delay was interposed between accurate responses and reinforcement. These differences were not seen when a variable delay was introduced. The difference between these two conditions may reflect the presence of trials with a zero delay in the unpredictable delay identical to reinforcement contingencies in all other tests of the SAT. When reinforcement was removed, compared to baseline performance, all females showed an initial (block 1) increase in accuracy for signal trials, but this benefit did not persist in blocks 2 and 3. All males showed an ability to maintain accuracy for signal trials when reinforcement was removed across all blocks. However, the

SHAM-LX, but not the DA-LX rats showed impaired non-signal performance in this session. While further examination is needed, the data suggest that DA-LX subjects may have nuanced sex differences in the ability to update reinforcement contingencies and that the deficits seen at the reversal stages of the ASST likely reflect an inability to update reinforcement contingencies in the face of distractors.

The SAT, unlike the ASST, can test separately susceptibility to distraction and specific alterations to reinforcement contingencies. Even on tests without the presence of distractors, DA-LX males were unable to consistently update reinforcement contingencies. DA-LX females were able to update reinforcement contingencies in a manner similar to SHAM-LX females. Together these data suggest that DA-LX males were less sensitive than all other subjects to changes in response-reinforcement timing.

One theory of the role of dopamine in the ACC is that it is a stability mechanism and that without it, complex representations are vulnerable to distraction from irrelevant stimuli (Robbins, 2005). In the ASST, reinforcement reversals require the ability to maintain attention on a specific dimension of a complex stimulus, and apply the reinforcement feedback to switch, not dimensions, but which of the attributes within that dimension should be selected (light foam shapes to dark foam shapes) (Alexander et al., 2012; Birrell & Brown, 2000; Tait & Brown, 2007). If there is a deficit in the stability mechanism to depress the irrelevant stimuli (lack of dopamine in the ACC) subjects may be slower to incorporate the information as they are continuing to select different stimuli dimensions, muddying the reinforcement feedback from the critic to the actor. When no distractors were present (SAT), all female DA-LX subjects showed an ability to update reinforcement contingencies, which may support the hypothesis that dopamine levels in the ACC are only necessary in the face of situations where reinforcement

history needs to be applied to specifically alter the signal-noise ratio in the DLPFC accordingly. If this is the case, we would expect that in situations where distractors are novel, reinforcement feedback can be utilized without the need for ACC involvement, possibly through the HRL-ACC actor-critic framework where the DLPFC can modify behavior based on trial-by-trial information, but is unable to make shifts based on more global task-sets (attributes of a complex stimulus) without the input from the ACC (Holroyd & Coles, 2002; Holroyd & Umemoto, 2016; Holroyd & Yeung, 2012). The idea that DA in the ACC provides a stable representation of the relevant stimulus dimension, which is then relayed to the DLPFC to selectively attend to that dimension, coheres with both presented roles of dopamine in the ACC.

Dopamine lesions revealed sex differences in the sustained attention task, but not the attentional set shifting task. These differences, while unexpected, were limited to specific differences in ability to update reinforcement contingencies. When DA-LX females were compared to SHAM-LX females, there was no difference in the ability of these animals to adjust response to delays in reinforcement or the complete removal of reinforcement. In contrast, DA-LX males were less sensitive than SHAM-LX males to delays in or removal of reinforcement. While neither the standard ASST or SAT have been shown to elicit sex differences (McGaughy et al., 1996; McGaughy & Sarter, 1999; Murphy et al., 2016), the dopamine system is sexually dimorphic in rodents (Duchesne, Dufresne, & Sullivan, 2009; Kritzer & Creutz, 2008), providing an anatomical basis for the illustrated behavioral differences.

The present study suggests a specific role for dopamine in the ACC within the HRL-ACC framework in conjunction with the hypothesis of dopamine as a stability mechanism. Both theories postulate that dopamine in the ACC is necessary to inhibit distractibility (Chudasama et al., 2003; Chudasama & Robbins, 2004; Holroyd & Umemoto, 2016; Robbins, 2005).

However, the present study suggests that dopamine in the ACC is only necessary to inhibit distraction when attributes of a complex stimulus have a prior reinforcement history. The HRL-ACC framework provides a role for dopamine in the ACC to aid in the integration of incoming stimuli and error feedback, which is used to select a task set (goal) (Holroyd & Umemoto, 2016). This task set is relayed to the DLPFC which recruits the necessary top-down control mechanism, and alterations in DA/NE levels to optimize the signal-noise level (Arnsten, 2011).

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APPENDICES

APPENDIX A

Tae Tables of Means and SEM form experiment 2 are presented in this appendix.

Table 2
Baseline Accuracy on Signal Trials For All Subjects

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline	67.38 ± 2.53	63.42 ± 1.76	68.14 ± 2.22	65.01 ± 2.37
500msec	88.77 ± 1.64	85.70 ± 0.77	87.27 ± 1.38	83.78 ± 1.19
100msec	70.86 ± 2.82	66.78 ± 2.40	71.45 ± 2.57	68.02 ± 5.03
25msec	42.51± 4.16	37.78 ± 2.93	45.69 ± 3.87	44.88 ± 3.30
Block 1	70.08 ± 2.49	65.27 ± 2.27	66.99 ± 3.84	68.92 ± 3.56
Block 2	66.26 ± 2.71	61.57 ± 2.36	69.18 ± 2.48	66.98 ± 2.66
Block 3	65.80 ± 2.87	63.43 ± 2.15	68.25 ± 2.73	60.71 ± 3.45
500msec block 1	89.83 ± 2.27	89.41 ± 1.17	86.69 ± 1.85	85.93 ± 2.79
500msec block 2	89.31 ± 2.30	85.66 ± 2.88	89.79 ± 1.76	86.18 ± 1.15
500msec block 3	87.16 ± 1.87	82.04 ± 1.99	82.34 ± 3.92	79.04 ± 3.62
100msec block 1	75.79 ± 0.32	70.03 ± 3.56	71.15 ± 4.19	72.31 ± 4.73
100msec block 2	69.06 ± 3.07	62.05 ± 2.60	72.09 ± 3.63	70.03 ± 4.31
100msec block 3	67.75 ± 3.44	68.27 ± 3.31	71.12 ± 4.15	61.60 ± 6.35
25msec block 1	44.61 ± 4.74	36.35 ± 3.89	40.14 ± 6.36	48.52 ± 4.91
25msec block 2	48.39 ± 3.86	46.04 ± 2.75	53.86 ± 3.02	52.66 ± 3.17
25msec block 3	42.50 ± 4.46	39.98 ± 2.60	51.28 ± 3.34	39.38 ± 5.23

Table 3
Baseline Non-Signal Accuracy for All Subjects

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline	85.16 ± 1.36	82.45 ± 1.36	83.54 ± 1.74	84.10 ± 1.56
Block 1	85.49 ± 1.53	84.13 ± 1.70	85.78 ± 1.46	84.68 ± 1.35
Block 2	87.18 ± 1.34	85.27 ± 0.91	87.08 ± 1.47	83.91 ± 1.87
Block 3	82.83 ± 2.02	77.94 ± 2.54	77.77 ± 3.47	83.70 ± 2.26

Table 4
Predictable Light Signal Trial Accuracy All Subjects

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	68.24 ± 3.20	61.37 ± 4.35	70.22 ± 3.06	61.26 ± 3.77
Plight Avg.	51.62 ± 3.45	44.62 ± 5.70	41.53 ± 3.80	36.78 ± 3.68
Baseline 500msec	89.07 ± 2.51	87.85 ± 2.54	85.46 ± 2.77	81.85 ± 3.14
Baseline 100msec	72.08 ± 3.42	59.55 ± 6.70	74.74 ± 2.78	66.08 ± 6.54
Baseline 25msec	43.56 ± 5.16	36.71 ± 5.53	50.46 ± 5.32	35.86 ± 4.68
Baseline Block 1	56.08 ± 2.50	51.04 ± 2.97	54.21 ± 3.74	43.25 ± 3.57
Baseline Block 2	47.92 ± 2.01	45.06 ± 4.41	52.97 ± 3.15	49.85 ± 3.56
Baseline Block 3	49.58 ± 3.38	41.97 ± 3.77	50.82 ± 4.40	44.74 ± 2.22
Baseline 500msec block 1	92.22 ± 2.37	91.67 ± 3.48	93.05 ± 3.60	84.12 ± 5.34
Baseline 500msec block 2	92.08 ± 3.35	84.37 ± 3.05	91.32 ± 3.69	86.57 ± 3.08
Baseline 500msec block 3	82.91 ± 5.46	87.50 ± 3.89	72.01 ± 9.20	74.92 ± 3.24
Baseline 100msec block 1	85.55 ± 3.33	68.06 ± 5.32	74.48 ± 7.30	63.49 ± 8.29
Baseline 100msec block 2	65.14 ± 3.87	56.59 ± 9.39	72.74 ± 5.89	73.81 ± 8.45
Baseline 100msec block 3	65.56 ± 6.51	53.99 ± 8.41	76.99 ± 6.52	60.95 ± 5.26
Baseline 25msec block 1	46.53 ± 8.49	44.44 ± 7.27	49.31 ± 9.11	25.40 ± 7.16
Baseline 25msec block 2	34.44 ± 5.09	39.29 ± 8.92	47.82 ± 6.85	39.08 ± 7.65
Baseline 25msec block 3	49.72 ± 6.12	26.39 ± 7.25	54.26 ± 6.74	43.08 ± 6.23
500msec	69.40 ± 4.50	59.61 ± 7.39	54.82 ± 4.63	43.72 ± 6.50
100msec	49.86 ± 4.43	35.13 ± 4.76	41.90 ± 4.23	34.37 ± 4.58
25msec	35.60 ± 3.53	39.12 ± 7.13	27.86 ± 4.26	32.36 ± 3.35
Block 1	55.09 ± 4.56	55.90 ± 7.18	48.90 ± 4.98	37.03 ± 3.33
Block 2	46.62 ± 4.50	43.06 ± 7.63	37.81 ± 5.84	31.88 ± 6.64
Block 3	53.14 ± 4.26	34.90 ± 5.00	37.86 ± 4.16	41.44 ± 6.57
500msec block 1	75.56 ± 6.98	68.75 ± 1.12	59.03 ± 6.72	49.20 ± 5.34
500msec block 2	64.72 ± 5.63	6.11 ± 9.62	50.79 ± 6.54	36.71 ± 9.15
500msec block 3	67.92 ± 4.87	48.96 ± 6.76	54.64 ± 6.70	45.24 ± 10.61
100msec block 1	54.17 ± 6.19	47.57 ± 5.64	43.75 ± 6.58	31.74 ± 4.49
100msec block 2	42.92 ± 5.52	36.81 ± 7.92	42.16 ± 8.25	30.36 ± 8.34
100msec block 3	52.50 ± 6.28	21.00 ± 7.37	39.78 ± 4.55	41.01 ± 6.84
25msec block 1	35.56 ± 5.62	51.39 ± 8.38	43.92 ± 6.05	30.16 ± 7.16
25msec block 2	32.22 ± 6.72	31.25 ± 7.42	20.48 ± 6.22	28.57 ± 6.78
25msec block 3	39.03 ± 5.67	34.72 ± 10.99	19.16 ± 5.98	38.06 ± 6.46

Table 5
Predictable Light Accuracy for Non-Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	83.56 ± 1.76	80.58 ± 2.54	80.54 ± 3.09	84.97 ± 2.60
P Light Avg.	76.86 ± 2.93	72.39 ± 5.13	74.15 ± 4.56	78.36 ± 3.23
Baseline Block 1	86.09 ± 1.95	75.30 ± 4.93	81.82 ± 4.48	89.42 ± 2.05
Baseline Block 2	87.25 ± 1.98	84.32 ± 2.97	84.85 ± 2.75	86.03 ± 2.19
Baseline Block 3	77.25 ± 3.53	82.11 ± 2.83	74.95 ± 5.08	79.46 ± 4.58
Plight Block 1	68.81 ± 3.64	64.52 ± 6.42	66.22 ± 4.34	70.47 ± 3.85
Plight Block 2	85.24 ± 3.90	74.49 ± 6.35	79.15 ± 5.14	82.53 ± 2.27
Plight Block 3	76.54 ± 4.01	78.15 ± 4.75	77.06 ± 6.31	82.08 ± 5.07

Table 6
Predictable Tone Accuracy for Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 6
Baseline Avg.	70.93 ± 3.32	67.45 ± 3.04	71.06 ± 2.56	61.25 ± 2.02
Ptone Avg.	69.42 ± 3.56	66.72 ± 2.60	57.17 ± 2.09	58.26 ± 3.18
Baseline 500msec	90.23 ± 1.85	86.17 ± 2.45	90.58 ± 2.43	81.63 ± 2.06
Baseline 100msec	75.48 ± 3.88	74.40 ± 4.38	68.86 ± 3.63	61.58 ± 6.41
Baseline 25msec	47.08 ± 6.00	41.77 ± 4.92	53.73 ± 5.20	40.54 ± 2.73
Baseline Block 1	71.46 ± 3.84	64.70 ± 4.59	68.34 ± 4.61	69.52 ± 3.74
Baseline Block 2	69.86 ± 4.05	67.13 ± 5.71	72.59 ± 5.47	62.82 ± 2.81
Baseline Block 3	71.48 ± 4.78	70.51 ± 4.26	72.24 ± 1.81	51.40 ± 2.04
Baseline 500msec block 1	85.14 ± 4.13	83.33 ± 4.69	94.44 ± 2.97	85.19 ± 5.49
Baseline 500msec block 2	91.11 ± 3.22	88.89 ± 5.56	93.75 ± 2.44	87.67 ± 4.34
Baseline 500msec block 3	94.44 ± 2.48	86.28 ± 6.01	83.53 ± 7.24	72.04 ± 7.65
Baseline 100msec block 1	80.48 ± 4.18	76.04 ± 6.45	67.53 ± 4.21	71.53 ± 8.35
Baseline 100msec block 2	73.75 ± 4.78	70.83 ± 7.25	72.12 ± 6.63	56.26 ± 6.87
Baseline 100msec block 3	72.22 ± 5.79	76.32 ± 5.74	66.92 ± 5.22	56.97 ± 9.69
Baseline 25msec block 1	48.75 ± 8.31	34.72 ± 7.40	43.06 ± 9.92	51.85 ± 6.83
Baseline 25msec block 2	44.72 ± 7.69	41.67 ± 7.20	51.88 ± 9.71	44.56 ± 7.39
Baseline 25msec block 3	47.78 ± 8.12	48.93 ± 5.49	66.25 ± 6.02	25.20 ± 6.94
500msec	88.32 ± 3.63	91.71 ± 1.60	81.23 ± 3.95	79.81 ± 4.17
100msec	73.43 ± 4.25	70.44 ± 3.19	57.50 ± 3.61	65.75 ± 6.12
25msec	46.53 ± 5.26	38.03 ± 5.26	32.80 ± 3.26	29.22 ± 4.27
Block 1	73.18 ± 3.85	68.98 ± 2.88	56.02 ± 2.75	62.87 ± 5.77
Block 2	67.36 ± 4.06	65.28 ± 3.20	55.62 ± 3.52	60.28 ± 5.37
Block 3	67.73 ± 4.02	65.91 ± 3.84	59.89 ± 4.23	51.62 ± 3.58
500msec block 1	86.34 ± 5.44	94.44 ± 2.97	85.32 ± 4.37	81.48 ± 8.45
500msec block 2	94.44 ± 2.48	93.06 ± 3.59	74.65 ± 6.60	79.17 ± 7.31
500msec block 3	84.17 ± 5.83	87.62 ± 2.22	83.72 ± 5.34	78.78 ± 6.25
100msec block 1	78.89 ± 4.52	75.00 ± 5.03	51.39 ± 4.67	67.46 ± 5.79
100msec block 2	68.33 ±6.73	72.22 ± 5.93	60.81 ± 6.59	70.19 ± 8.49
100msec block 3	73.05 ± 6.23	64.09 ± 5.41	60.28 ± 6.60	59.59 ± 10.55
25msec block 1	54.31 ± 7.78	37.50 ± 3.60	31.35 ± 2.54	39.68 ± 9.90
25msec block 2	39.31 ± 6.22	30.56 ± 6.88	31.39 ± 3.67	31.48 ± 5.49
25msec block 3	45.97 ± 7.45	46.03 ± 8.22	35.65 ± 7.80	16.50 ± 7.57

Table 7
Predictable Tone Accuracy for Non-Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 6
Baseline Avg.	85.34 ± 2.17	86.02 ± 1.94	77.77 ± 3.59	83.54 ± 1.79
P Tone Avg.	84.19 ± 2.81	83.99 ± 1.76	79.00 ± 4.63	87.79 ± 3.00
Baseline Block 1	84.72 ± 2.35	89.16 ± 1.30	79.60 ± 3.01	83.10 ± 4.30
Baseline Block 2	88.00 ± 2.40	88.28 ± 3.00	83.71 ± 4.16	84.97 ± 2.42
Baseline Block 3	83.29 ± 4.73	80.63 ± 3.14	69.99 ± 7.32	82.56 ± 3.92
Ptone Block 1	83.06 ± 4.46	85.07 ± 2.19	83.53 ± 5.00	84.42 ± 5.48
PtoneBlock 2	83.27 ± 2.87	89.17 ± 2.84	76.24 ± 5.06	84.02 ± 4.26
Ptone Block 3	86.22 ± 2.01	77.73 ± 3.26	77.24 ± 6.89	95.47 ± 2.45

Table 8
Predictable Delay of Reinforcement Accuracy for Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	63.12 ± 4.61	60.84 ± 2.27	65.65 ± 3.25	63.69 ± 2.41
P Delay Avg.	64.01 ± 3.57	60.50 ± 4.31	57.72 ± 3.39	54.00 ± 3.85
Baseline 500msec	87.36 ± 3.02	84.08 ± 1.73	86.78 ± 3.50	79.03 ± 3.34
Baseline 100msec	57.18 ± 5.85	51.68 ± 5.03	59.94 ± 4.19	64.35 ± 4.96
Baseline 25msec	35.37 ± 5.52	37.56 ± 4.51	43.78 ± 6.58	44.40 ± 4.21
Baseline Block 1	65.60 ± 4.28	62.91 ± 4.65	65.74 ± 3.69	70.97 ± 3.66
Baseline Block 2	62.50 ± 4.60	58.16 ± 4.07	68.15 ± 3.03	66.20 ± 3.38
Baseline Block 3	61.25 ± 5.22	61.45 ± 4.35	63.06 ± 5.68	53.91 ± 6.98
Baseline 500msec block 1	91.11 ± 3.23	88.89 ± 3.64	90.28 ± 3.28	84.13 ± 4.10
Baseline 500msec block 2	85.56 ± 4.70	83.33 ± 5.14	91.32 ± 3.04	83.33 ± 2.32
Baseline 500msec block 3	85.52 ± 2.88	80.00 ± 6.74	78.73 ± 73.80	69.64 ± 8.72
Baseline 100msec block 1	69.58 ± 6.46	63.89 ± 8.59	66.67 ± 5.94	77.78 ± 5.94
Baseline 100msec block 2	65.28 ± 7.63	55.56 ± 5.56	66.34 ± 4.16	67.43 ± 9.02
Baseline 100msec block 3	65.00 ± 7.41	63.19 ± 7.70	66.17 ± 7.99	57.73 ± 9.42
Baseline 25msec block 1	36.11 ± 6.31	35.94 ± 5.34	40.28 ± 7.55	50.99 ± 5.29
Baseline 25msec block 2	36.67 ± 5.25	35.59 ± 5.22	46.80 ± 5.86	47.85 ± 4.74
Baseline 25msec block 3	33.33 ± 8.28	41.15 ± 6.72	44.27 ± 9.86	34.35 ± 11.29
500msec	80.26 ± 2.32	78.67 ± 5.31	73.78 ± 3.32	70.61 ± 4.43
100msec	68.64 ± 4.56	60.91 ± 6.12	60.67 ± 4.22	54.46 ± 6.06
25msec	43.14 ± 6.66	41.92 ± 4.52	38.72 ± 3.67	36.93 ± 3.81
Block 1	64.72 ± 4.44	56.54 ± 5.70	57.75 ± 6.01	55.42 ± 4.76
Block 2	66.87 ± 4.29	64.43 ± 3.86	55.37 ± 3.93	54.44 ± 6.77
Block 3	60.45 ± 4.61	60.54 ± 5.86	60.05 ± 4.57	52.14 ± 3.06
500msec block 1	80.97 ± 4.68	72.22 ± 6.96	73.61 ± 5.12	78.91 ± 4.98
500msec block 2	85.28 ± 3.70	86.70 ± 4.24	70.78 ± 6.15	72.78 ± 8.99
500msec block 3	74.54 ± 5.82	77.08 ± 8.94	76.93 ± 5.96	60.15 ± 7.40
100msec block 1	7.22 ± 5.97	57.81 ± 8.27	58.32 ± 9.04	59.72 ± 5.26
100msec block 2	67.28 ± 6.88	67.71 ± 7.04	58.85 ± 4.35	57.73 ± 10.76
100msec block 3	66.41 ± 7.08	57.22 ± 8.86	64.83 ± 6.40	45.92 ± 8.99
25msec block 1	40.97 ± 8.24	39.58 ± 7.66	41.32 ± 6.50	27.63 ± 7.84
25msec block 2	48.06 ± 8.20	38.86 ± 4.43	36.46 ± 3.90	32.81 ± 4.12
25msec block 3	40.39 ± 6.86	47.32 ± 9.75	38.39 ± 9.86	50.36 ± 5.85

Table 9
Predictable Delay of Reinforcement for Non-signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	86.18 ± 2.22	83.07 ± 1.02	84.33 ± 2.98	86.63 ± 2.79
P Delay Avg.	77.82 ± 2.33	82.16 ± 1.61	84.41 ± 1.71	83.41 ± 2.95
Baseline Block 1	86.58 ± 2.58	86.27 ± 1.27	88.37 ± 2.37	85.12 ± 3.69
Baseline Block 2	88.61 ± 2.62	83.71 ± 1.86	86.74 ± 2.83	89.68 ± 3.16
Baseline Block 3	83.33 ± 2.90	79.24 ± 2.98	77.88 ± 6.59	85.08 ± 4.32
PDelay Block 1	79.89 ± 3.57	85.75 ± 2.84	87.36 ± 2.66	82.09 ± 5.85
PDelay Block 2	76.72 ± 2.40	76.45 ± 2.41	85.34 ± 2.23	85.97 ± 2.74
PDelay Block 3	76.86 ± 3.36	84.27 ± 2.37	80.54 ± 5.21	82.17 ± 5.09

Table 10
Unpredictable Delay of Reinforcement Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	65.44 ± 3.38	66.69 ± 3.10	66.94 ± 2.28	67.15 ± 3.61
U Delay Avg.	67.10 ± 3.20	69.51 ± 4.25	59.23 ± 3.16	63.10 ± 4.05
Baseline 500msec	85.32 ± 2.68	88.83 ± 2.12	88.21 ± 1.61	85.36 ± 2.57
Baseline 100msec	66.94 ± 4.67	72.92 ± 3.64	73.93 ± 3.33	73.35 ± 5.63
Baseline 25msec	44.07 ± 4.91	39.23 ± 5.26	38.70 ± 4.31	42.74 ± 7.00
Baseline Block 1	68.94 ± 3.41	66.09 ± 4.23	65.74 ± 3.69	73.81 ± 5.54
Baseline Block 2	65.19 ± 4.88	64.24 ± 2.51	66.77 ± 4.33	67.34 ± 3.42
Baseline Block 3	62.21 ± 3.95	70.65 ± 5.05	68.33 ± 3.55	60.30 ± 4.54
Baseline 500msec block 1	90.69 ± 3.38	93.06 ± 2.92	90.28 ± 3.28	88.89 ± 4.20
Baseline 500msec block 2	85.56 ± 4.07	87.33 ± 2.51	86.63 ± 3.74	83.08 ± 3.76
Baseline 500msec block 3	79.70 ± 5.54	86.11 ± 4.07	87.71 ± 5.40	84.12 ± 7.59
Baseline 100msec block 1	70.00 ± 6.64	70.66 ± 7.50	76.39 ± 4.42	81.94 ± 6.37
Baseline 100msec block 2	67.78 ± 6.92	67.88 ± 4.31	71.48 ± 7.72	78.17 ± 6.12
Baseline 100msec block 3	63.04 ± 4.05	80.21 ± 4.62	73.91 ± 6.66	59.92 ± 9.96
Baseline 25msec block 1	46.11 ± 3.52	34.55 ± 6.52	30.56 ± 7.20	50.59 ± 13.15
Baseline 25msec block 2	42.22 ± 7.55	37.50 ± 7.25	42.19 ± 7.13	40.77 ± 8.32
Baseline 25msec block 3	43.89 ± 7.61	45.63 ± 8.02	43.37 ± 6.28	36.84 ± 6.73
500msec	85.60 ± 3.54	87.79 ± 3.87	83.07 ± 3.74	82.83 ± 3.71
100msec	69.85 ± 4.93	73.69 ± 4.55	59.54 ± 6.88	67.66 ± 6.14
25msec	45.86 ± 4.15	47.04 ± 5.49	35.09 ± 4.91	38.81 ± 5.07
Block 1	71.11 ± 3.05	65.28 ± 5.81	64.70 ± 3.14	65.18 ± 4.36
Block 2	65.19 ± 4.06	71. 64 ± 3.27	60.57 ± 4.10	63.54 ± 4.00
Block 3	65.01 ± 4.39	71.60 ± 6.07	52.42 ± 4.28	60.57 ± 5.13
500msec block 1	92.08 ± 2.91	83.33 ± 6.96	84.72 ± 5.92	83.67 ± 7.18
500msec block 2	82.92 ± 4.65	91.67 ± 3.48	91.67 ± 3.48	80.56 ± 5.85
500msec block 3	81.81 ± 5.18	88.37 ± 5.06	72.81 ± 6.46	84.24 ± 5.27
100msec block 1	74.72 ± 5.68	73.61 ± 5.92	70.49 ± 4.10	69.25 ± 7.22
100msec block 2	70.97 ± 4.97	74.65 ± 5.02	56.87 ± 9.32	66.30 ± 8.38
100msec block 3	63.85 ± 7.58	72.82 ± 8.36	5.13 ± 9.40	67.43 ± 7.11
25msec block 1	46.53 ± 4.28	38.89 ± 6.96	38.89 ± 4.70	42.63 ± 6.60
25msec block 2	41.67 ± 7.82	48.61 ± 5.53	33.16 ± 6.44	43.76 ± 7.86
25msec block 3	49.38 ± 4.17	53.62 ± 8.42	33.21 ± 8.23	30.03 ± 6.79

Table 11
Unpredictable Delay of Reinforcement Accuracy on Non-Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	83.73 ± 2.07	81.77 ± 2.14	86.41 ± 2.44	83.25 ± 2.53
U Delay Avg.	82.70 ± 2.15	80.56 ± 1.45	83.28 ± 1.62	81.12 ± 2.04
Baseline Block 1	82.62 ± 2.69	89.75 ± 1.97	89.33 ± 2.36	85.19 ± 3.33
Baseline Block 2	85.31 ± 2.93	85.89 ± 2.12	88.72 ± 1.80	74.22 ± 4.20
Baseline Block 3	83.26 ± 2.90	69.69 ± 4.79	81.17 ± 5.48	90.33 ± 2.64
UDelay Block 1	86.74 ± 3.27	85.47 ± 2.10	86.21 ± 2.88	79.19 ± 4.85
UDelay Block 2	82.51 ± 2.38	85.49 ± 3.08	86.32 ± 2.50	85.80 ± 2.61
UDelay Block 3	78.86 ± 2.45	70.71 ± 3.27	77.31 ± 3.11	78.39 ± 4.15

Table 12
Removal of Reinforcement Accuracy on Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	73.90 ± 3.57	61.38 ± 2.71	66.82 ± 4.52	71.13 ± 3.41
Removal Avg.	68.31 ± 2.56	63.10 ± 4.43	63.67 ± 3.36	61.74 ± 3.54
Baseline 500msec	95.88 ± 1.54	84.43 ± 1.84	85.34 ± 2.43	89.53 ± 2.85
Baseline 100msec	75.60 ± 4.80	65.16 ± 5.87	73.34 ± 5.51	73.90 ± 5.33
Baseline 25msec	50.23 ± 5.75	34.55 ± 4.98	41.78 ± 7.47	49.96 ± 5.82
Baseline Block 1	77.78 ± 3.83	61.52 ± 4.54	62.85 ± 6.78	70.37 ± 3.23
Baseline Block 2	74.31 ± 4.87	56.88 ± 4.48	67.77 ± 4.85	71.30 ± 3.86
Baseline Block 3	69.63 ± 4.61	65.75 ± 5.14	69.85 ± 3.76	71.72 ± 4.91
Baseline 500msec block 1	96.67 ± 1.70	87.33 ± 5.72	80.38 ± 4.02	92.06 ± 3.99
Baseline 500msec block 2	95.56 ± 2.46	84.72 ± 3.60	85.94 ± 3.47	90.48 ± 4.49
Baseline 500msec block 3	95.42 ± 1.88	81.25 ± 8.06	89.71 ± 2.37	86.05 ± 4.11
Baseline 100msec block 1	82.22 ± 5.29	66.67 ± 7.86	70.66 ± 9.56	68.25 ± 5.65
Baseline 100msec block 2	76.67 ± 6.92	58.33 ± 6.55	77.78 ± 5.94	80.56 ± 5.71
Baseline 100msec block 3	67.92 ± 8.14	70.49 ± 9.35	71.58 ± 7.84	72.88 ± 9.26
Baseline 25msec block 1	54.44 ± 7.11	30.56 ± 6.56	37.50 ± 10.27	50.79 ± 8.69
Baseline 25msec block 2	50.69 ± 8.40	27.58 ± 6.90	39.58 ± 9.36	42.86 ± 6.61
Baseline 25msec block 3	45.56 ± 6.30	45.52 ± 6.21	48.26 ± 6.43	56.24 ± 9.55
500msec	78.66 ± 6.30	73.90 ± 4.11	76.86 ± 2.96	80.12 ± 4.46
100msec	72.51 ± 4.35	61.22 ± 5.45	68.06 ± 3.56	59.28 ± 4.96
25msec	53.76 ± 3.88	54.19 ± 5.59	46.09 ± 7.76	45.82 ± 2.78
Block 1	73.82 ± 4.79	70.83 ± 3.80	75.64 ± 3.01	71.61 ± 2.73
Block 2	65.39 ± 5.52	59.89 ± 2.16	62.58 ± 2.66	54.06 ± 6.76
Block 3	65.72 ± 4.96	58.59 ± 9.13	52.79 ± 7.08	59.56 ± 3.33
500msec block 1	91.59 ± 4.04	88.89 ± 2.97	92.88 ± 2.09	86.25 ± 1.47
500msec block 2	78.30 ± 5.57	73.26 ± 5.07	71.78 ± 6.10	75.28 ± 7.66
500msec block 3	66.10 ± 5.68	59.55 ± 7.33	65.92 ± 7.01	78.83 ± 5.70
100msec block 1	78.47 ± 7.79	73.61 ± 6.28	78.47 ± 4.48	75.00 ± 5.76
100msec block 2	65.67 ± 7.96	57.81 ± 4.04	68.75 ± 6.45	45.26 ± 7.78
100msec block 3	73.38 ± 5.81	52.22 ± 1.37	56.96 ± 6.41	57.59 ± 5.02
25msec block 1	51.39 ± 5.02	50.00 ± 8.66	55.56 ± 8.66	53.57 ± 4.47
25msec block 2	52.20 ± 6.71	48.58 ± 3.25	47.22 ± 6.88	41.64 ± 7.59
25msec block 3	57.69 ± 8.74	63.99 ± 9.99	35.49 ± 10.65	42.26 ± 5.15

Table 13
Removal of Reward Non-Signal Trials

	Sham-LX Male n = 10	DA-LX Male n = 8	Sham-LX Female n = 8	DA-LX Female n = 7
Baseline Avg.	86.12 ± 1.88	85.57 ± 2.45	88.66 ± 2.27	82.11 ± 1.86
Removal Avg.	76.17 ± 2.62	81.87 ± 2.55	80.04 ± 1.96	77.60 ± 3.12
Baseline Block 1	84.07 ± 3.54	89.81 ± 2.29	89.78 ± 2.49	82.01 ± 2.05
Baseline Block 2	87.29 ± 2.37	86.41 ± 2.79	91.35 ± 3.09	84.67 ± 2.16
Baseline Block 3	86.99 ± 1.78	80.50 ± 4.01	84.84 ± 2.81	79.64 ± 4.67
Removal Block 1	83.02 ± 2.31	84.72 ± 3.46	85.75 ± 2.59	81.15 ± 1.70
Removal Block 2	76.11 ± 2.76	78.78 ± 5.13	82.97 ± 3.01	80.90 ± 4.67
Removal Block 3	69.39 ± 5.02	82.10 ± 4.79	71.39 ± 3.93	70.75 ± 4.31

APPENDIX B

For all experiments reported in this thesis, approval for the use of animal subjects was obtained from the University of New Hampshire Institutional Animal Care and Use Committee (IACUC). Forms demonstrating proof of the approval are included in this appendix.

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

15-Nov-2019

McGaughy, Jill A
Psychology
McConnell Hall Rm 404F
Durham, NH 03824-2602

IACUC #: 181203
Project: Attentional Effects of Lesions to the Anterior Cingulate Cortex (Part 2)
Next Review Date: 13-Dec-2020

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your request for a time extension for this protocol. Approval is granted until the "Next Review Date" indicated above. You will be asked to submit a report with regard to the involvement of animals in this study before that date. If your study is still active, you may apply for extension of IACUC approval through this office.

The appropriate use and care of animals in your study is an ongoing process for which you hold primary responsibility. Changes in your protocol must be submitted to the IACUC for review and approval prior to their implementation.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Susan Jalbert at 862-3536.

For the IACUC,

Julie Simpson, Ph.D.
Director

cc: File

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

14-Dec-2018

McGaughy, Jill A
Psychology
McConnell Hall Rm 404F
Durham, NH 03824-2602

IACUC #: 181203
Project: Attentional Effects of Lesions to the Anterior Cingulate Cortex (Part 2)

The Institutional Animal Care and Use Committee (IACUC) has reviewed and recommended approval of the protocol submitted for this study contingent upon your response to the following:

1. *The investigator needs to submit page 1 of the application with the required signatures.*
2. *In Section II, E of the application, the investigator needs to add Euthasol as a controlled substance.*
3. *The investigator needs to provide information in all parts for Section VII, A, #s 5, 6, 7, and 12.*
4. *The investigator needs to provide the number of animals in #2 of the Surgical Procedures Form.*

As soon as the IACUC receives an appropriate response to its concerns, above, it will issue you an approval letter for this protocol. **You may not commence activities in this protocol involving vertebrate animals until you have received IACUC approval.**

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Rebecca Rowe

Rebecca Rowe, Ph.D.
Chair

cc: File

University of New Hampshire

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Application for Vertebrate Animal Use in Research

Principal Investigator: Jill A. McGaughy

Project Title: Attentional effects of lesions to the anterior cingulate cortex

Proposed Start Date: January 2, 2019 Anticipated Completion Date: January 2, 2022

SECTION I: INVESTIGATOR ASSURANCE FOR HUMANE CARE AND USE OF ANIMALS IN RESEARCH

I, the Principal Investigator named above, certify that:

- The information included in this application is complete and accurate to the best of my knowledge.
- All personnel listed recognize and agree to accept their responsibility in complying with the PHS Policy for the Humane Care and Use of Laboratory Animals, USDA rules and University of New Hampshire policies governing the care and use of animals in research.
- All personnel listed will comply with DEA, Occupational Health and Safety, and Biohazard regulations.
- All procedures involving live animals will be performed under my supervision or that of another qualified individual identified in this application.
- Procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project will be carried out by properly trained and qualified personnel.
- If this project is funded by an extramural source, this application accurately reflects all procedures involving laboratory animal subjects described in the proposal to the funding source.
- Prior to implementing any revisions or variations from the approved animal care and use protocol, I will submit proposed changes, in writing, to the Institutional Animal Care and Use Committee (IACUC) for review and approval.
- Where applicable, I conducted the literature search (in Section V) and found no alternatives to the potentially painful/distressful procedure(s) outlined in this protocol and that the keywords used were directed at finding alternatives to the potentially painful/ distressful procedure(s).
- I will provide continuing education to project personnel throughout the duration of the study, as appropriate (e.g., via direct supervision, during lab/staff meetings).
- In conducting this project, I will follow the IACUC-approved protocol and will only use IACUC-approved procedures.

Principal Investigator Signature	Date	Department Chairperson or Authorized Individual Signature	Date
Name (Typed or Printed)		Name (Typed or Printed)	

FOR IACUC USE ONLY

IACUC# 181203 Approval Date:

VETERINARIAN'S REVIEW:

Signature Date

Name (Typed or Printed)

Application: Original ___ Modified ___

a. Pre-Review Completed ___

b. Return for Revision ___

Comments: